DETERMINATION OF PYRAZINAMIDE PLASMA CONCENTRATIONS USING LC-MS AND PHARMACOKINETICS OF PYRAZINAMIDE IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS AND IN PATIENTS CO-INFECTED WITH MULTIDRUG-RESISTANT TUBERCULOSIS AND HIV

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THESIS SUBMITTED TO THE DISCIPLINE OF PHARMACOLOGY, SCHOOL OF PHARMACY, UNIVERSITY OF THE WESTERN CAPE, IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MAGISTER PHARMACEUTICAE.

OCTOBER 2013

Determination of pyrazinamide plasma concentrations using LC-MS and pharmacokinetics of pyrazinamide in patients with multidrug-resistant tuberculosis and in patients co-infected with multidrug-resistant tuberculosis and HIV

KEYWORDS:

Pyrazinamide

Pharmacokinetics

Multi drug-resistant tuberculosis

HIV/AIDS

Liquid chromatography-mass spectrometry

I, Carla Ilse Botha, declare that *Determination of pyrazinamide plasma concentrations using LC-MS and pharmacokinetics of pyrazinamide in patients with multidrug-resistant tuberculosis and in patients co-infected with multidrug-resistant tuberculosis and HIV*, is my own work, that it has not been submitted for any degree or examination to any other University, and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

Signed:

Carla Ilse Botha

Date:

27/2/2014

ACKNOWLEDGMENTS:

Prof P Mugabo – for his patience, support and faith in me

Prof R Madsen – for his assistance with the statistical analysis of our results

Dr D Theron, staff and patients at Brewelskloof Hospital, Worcester – for their assistance and participation in this study

Dr M Stander and staff at Central Analytical Facility, University of Stellenbosch – for assistance with the assays of our research samples

My brother, mother and Late father – for their never ending support and encouragement

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3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ALT	Alanine transaminase
ARVs	Antiretrovirals
AST	Aspartate aminotransferase
AZT	Zidovudine
CNS	Central nervous system
CSF	Cerebrospinal fluid
d4T	Stavudine
DNA	Deoxyribonucleic acid
DOH	South African Department of Health
DS-TB	Drug sensitive/susceptible tuberculosis

EMB	Ethambutol
EFV	Efavirenz
FTC	Emtricitabine
GC	Gas Cromatography
GGT	Gamma-glutamyl transferase
GFR	Glomerular filtration rate
GIT	Gastro-intestinal tract
HIV	Human Immunodeficiency Virus
HIVAN	HIV associated nephropathy
HPLC	High Performance Liquid Chromatography
IM(I)	Intramuscular (injection)
INH	Isoniazid
IPT	Isoniazid Preventative Therapy
IRIS	Immune reconstitution inflammatory syndrome
IV(I)	Intravenous (injection)

LC-MS	Liquid chromatography-mass spectrometry
MDR-TB	Multi drug-resistant tuberculosis
MIC	Minimum inhibitory concentration
MRM	Multiple reaction mode
MSF	Médecins Sans Frontières (Doctors Without Borders)
MTB	*Mycobacterium tuberculosis*
NNRTIS	Non-nucleoside reverse transcriptase inhibitors
NRTIS	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PAS	Para-aminosalicylic acid
PIs	Protease inhibitors
PZA	Pyrazinamide
RIF	Rifampicin
RNA	Ribonucleic acid

TB	Tuberculosis
TBM	TB Meningitis
TDF	Tenofovir
TDR-TB	Totally drug-resistant tuberculosis
TDM	Therapeutic Drug Monitoring
UPLC	Ultra-performance liquid chromatography
WFA	Percentage of Expected Weight for age (Wellcome Classification)
WHO	World Health Organisation
XDR-TB	Extensively drug-resistant tuberculosis

ABSTRACT:

Tuberculosis and HIV are arguably South Africa's largest and most important health issues. With drug-resistant strains of tuberculosis on the increase and little research on new drugs, there is an urgent need for research around the drugs presently available to ensure their optimal use and to minimise their sometimes serious and significant side effects.

Treatment of drug-resistant tuberculosis is expensive and lengthy, and is complicated by a limited choice of drugs with lower efficacies and higher toxicities. Treatment is further complicated in patients with HIV due to several factors including drug interactions. While some authors suggest that HIV and malabsorption might be associated with poor clinical outcomes, other researchers have found no link. Patients may benefit from Therapeutic Drug Monitoring in order to ensure that their doses of antituberculosis drugs are reaching the required minimum effective concentrations, without attaining toxic levels in the plasma which may cause unpleasant side effects. There is little research concerning drug levels in HIV patients with TB in South Africa, let alone in patients with drug-resistant forms of tuberculosis, and there are no studies in this country that use Liquid Chromatography-Mass Spectrometry to investigate the plasma levels of pyrazinamide in patients with MDR-TB.

This study aimed to investigate whether or not there is a difference in the pharmacokinetics of PZA in MDR-TB patients with HIV, and those without HIV infection. It also aimed to establish whether LC-MS could be used to study the levels of pyrazinamide in the plasma of patients with multidrug-resistant tuberculosis with and without concurrent HIV infection. The plasma levels of pyrazinamide in 32 MDR-TB patients (23 HIV negative and 9 HIV positive), were successfully

analysed using LC-MS, and the pharmacokinetics of PZA in these 2 populations was described. It was established that the T_{max} of pyrazinamide was significantly higher in HIV-negative patients than in HIV-positive patients. Although there was a difference between the K_a in the two populations, this difference did not quite reach statistical significance. There were no statistically significant differences between HIV-negative and HIV-positive patients with regards to the other pharmacokinetic parameters investigated.

Our findings established that there was little evidence to suggest that there is a difference between the pharmacokinetics of the antimycobacterial drug pyrazinamide in HIV-positive patients and that in HIV-negative patients. We were also able to successfully develop and validate an assay for the analysis of PZA in plasma using LC-MS, and this finding could be very valuable for further studies.

Although our study failed to prove this, the possibility still exists that HIV-positive patients could exhibit altered kinetics of antiTB drugs and this has not been fully investigated in South Africa. The clinical impact of low plasma levels of antimycobacterial drugs is still largely unexplored and further research with larger sample sizes should be done in order to establish which factors may contribute to low plasma levels of anti-tuberculosis drugs in MDR-TB patients, and whether or not these low levels are increasing the risk of treatment failure or other poor clinical outcomes.

CHAPTER ONE:

INTRODUCTION

Tuberculosis (TB) is an ancient disease that is reappearing at increasingly alarming rates, especially in South Africa. Even more concerning, is the emergence of Multidrug-Resistant Tuberculosis (MDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB).

Recently, researchers in India described patients with a terrifying new disease: Totally Drug-Resistant Tuberculosis (TDR-TB), where patients are resistant to all available anti-tuberculosis treatments [Udwadia et al, 2012]. Similar cases were reported in Iran by Velayati et al [2009:420]. While cure rates for drug-susceptible tuberculosis are generally high - 79% in 2010 in South Africa [WHO, 2012_a], the estimated cure rate for MDR-TB worldwide in 2009 was only around 48% [WHO, 2013_a]. The cure rate for MDR-TB in South Africa is also estimated to be less than 50% [DOH, 2009_a:ii].

Currently, according to the South African Department of Health (DOH) guidelines, pyrazinamide (PZA) forms part of the regimen for the treatment of both drug-susceptible tuberculosis (DS-TB) and MDR-TB [DOH, 2009_b; DOH, 2011]. It is used together with other antimycobacterial drugs, as resistance develops rapidly when used as monotherapy.

There are several studies concerning the pharmacokinetics of PZA in patients with and without tuberculosis, but PZA's pharmacokinetics in HIV (human immunodeficiency virus) positive patients is not fully described [Perlman et al, 2004], and there are seemingly no studies in South

Africa that demonstrate the pharmacokinetics in patients with drug-resistant TB, and specifically drug-resistant TB and HIV infection.

The aim of this study was to use liquid chromatography-mass spectrometry (LC-MS) to investigate PZA plasma concentrations, to describe the pharmacokinetics of PZA in patients with MDR-TB and in those with MDR-TB and HIV infection, and to see if there may be differences between the groups. This was achieved by obtaining blood samples from 32 MDR-TB patients both with, and without HIV, and using LC-MS to analyse the levels of PZA within these samples. This information was then used to conduct pharmacokinetic analysis of the 2 patient groups, and the results were compared statistically.

Information gathered in this study could shed new light on the characteristics of pyrazinamide pharmacokinetics in MDR-TB patients and give a better understanding of whether or not these patients may be subject to subtherapeutic drug levels. This information could be especially valuable in patients with HIV infection who may be more susceptible to decreased serum concentrations of anti-TB drugs, possibly due to impaired absorption [Yew, 2001; Li et al, 2004; Peloquin, 1998] or who may be taking antiretroviral drugs which may interact with, or alter the pharmacokinetics of antimycobacterial drugs. [Nuermberger and Grosset, 2004; Coyne et al, 2009; de Jong et al, 2004].

CHAPTER TWO:

LITERATURE REVIEW

2.1 Tuberculosis:

2.1.1 History, epidemiology, and symptoms

Tuberculosis is an infectious disease caused by the bacterium *Mycobacterium tuberculosis.* The tubercule bacillus was first discovered by Nobel Laureate Robert Koch in 1882, and while it typically infects the lungs, it may infect almost any area in the body. The infection is spread by the inhalation of bacteria in small droplet nuclei which become airborne when a TB patient coughs, sneezes or talks. Common symptoms include chronic cough, fever, night sweats and weight loss [WHO, 2013_b].

Mycobacterium tuberculosis (MTB) has killed more people than any other single infection in history, and there is ever increasing drug resistance, yet few new drugs are being brought onto the market to treat these resistant strains. In the 1970's and 1980's, TB rates in the USA were declining, and so funding for research was cut drastically. TB rates then started to increase, with many cases of extra-pulmonary TB appearing. This was as a result of the rapidly emerging HIV/AIDS pandemic. A study presented at the annual joint meeting of The American Thoracic Society and The American Lung Association in 1984 reported that: "this new disease 'AIDS' was "fueling the latent fire of a familiar old infection" [in Reichman and Hopkins Tanne, 2002].

TB caused 1,4 million deaths worldwide in 2011 alone [WHO, 2013_b], with 25000 South Africans dying of the disease in 2011 [WHO, 2012_a]. South Africa recorded 325321 new cases of TB and 45915 re-treatment cases in 2011; the cure rate for re-treatment TB in 2010 was only 35% [WHO, 2012_a].

2.1.2. Treatment:

Treatment for DS-TB in South Africa consists of chemotherapy for six months with a combination of four oral antimycobacterial drugs, namely; rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (EMB). This consists of an intensive phase of RIF, INH, PZA and EMB for 2 months followed by a continuation phase of RIF and INH for 4 months. In retreatment cases, therapy may also include the use of the aminoglycoside streptomycin by intramuscular injection [DOH, 2009_b].

2.2 Multi-drug resistant tuberculosis:

2.2.1 Definition:

MDR-TB is defined as TB that is resistant to rifampicin and isoniazid, with or without resistance to other antimycobacterial drugs [DOH, 2009_b; Sharma et al, 2006].

2.2.2 History and Epidemiology:

According to the World Health Organisation, there were an estimated 500 000 new MDR-TB cases worldwide in 2011, around 3.7% of new TB cases were MDR while around 20% of re-treatment cases were classified as MDR-TB. Approximately 60% of the world's MDR-TB cases occur in the following countries: Brazil, China, India, the Russian Federation and South Africa [WHO, 2013a].

With the cost of treating MDR-TB at about 25 times higher than that of DS-TB [Pooran et al, 2013], and cure rates as low as 48% [WHO, 2013_a]. MDR-TB is a health crisis with far-reaching economic and social consequences.

There are two mechanisms by which a patient may acquire MDR-TB; the first is primary resistance whereby a patient who has not previously been treated for TB becomes infected with a drug-resistant strain of *Mycobacterium tuberculosis*, the second is acquired resistance, where a patient has been treated for TB on one or more occasions and hence where resistance is likely to have been caused by an insufficient amount of one or more antimycobacterial drugs [Friedland, 2007_a]. This insufficient amount or improper use may be caused by poor patient compliance, poor quality medicines, or the administration of inappropriate treatment regimens, for example in areas with poor TB control programs [WHO, 2013_b]. Inadequate drug levels select for resistant mutants, and once patients have resistance to one of the drugs, they are more likely to develop resistance to other drugs too [Friedland, 2007_a]. Researchers in Kazakhstan identified alcoholism, smoking, increased number of previous TB treatments as well as irregular treatment, as being risk factors for acquired DR-TB [Barroso et al, 2003].

2.2.3 Treatment:

The treatment of drug-resistant TB is much more complicated than the treatment of drug-sensitive TB. It involves the use of drugs that are much more expensive, more toxic, less effective and less tolerable [WHO, 2013_a; Kahana, 1996]. It may also include the use of 1st line drugs to which the patient's isolate is still sensitive, for example PZA, ethambutol, or even isoniazid. MDR-TB cure rates are lower [WHO, 2013_a] and treatment far more expensive than that of DS-TB [MSF, 2011]. In addition to this, the duration of treatment is much longer, usually at least 18-24 months in MDR-TB as opposed to 6 months in drug-sensitive TB. In South Africa, the national guidelines [DOH, 2011] recommend a 5-drug regimen during a 6-month intensive phase followed by a 4-drug regimen during the18 month continuation phase. These regimens are shown below in Table 3 and Table 4. Although ideally these patients are hospitalized for at least part of their treatment, a lack of resources amongst other reasons means that some patients undergo "Clinic-based care" instead.

Table 1: Standardised National MDR-TB Treatment Regimen for Adults and Adolescents (Intensive phase) [DOH, 2011]. This is taken at least six days a week, for at least 6 months, guided by TB Culture Conversion

Patient Weight:	Drug:	Dose:
<33kg	Kanamycin	15-20mg/kg
	Moxifloxacin	400mg
	Ethionamide	15-20mg/kg

	Terizidone	15-20mg/kg
	Pyrazinamide	30-40mg/kg
33-50kg	Kanamycin	15-20mg/kg
	Moxifloxacin	400mg
	Ethionamide	500mg
	Terizidone	750mg
	Pyrazinamide	1000-1750mg
51-70kg	Kanamycin	1000mg
	Moxifloxacin	400mg
	Ethionamide	750mg
	Terizidone	750mg
	Pyrazinamide	1750-2000mg
>70kg	Kanamycin	1000mg
	Moxifloxacin	400mg
	Ethionamide	750-1000mg

	Terizidone	750-1000mg
	Pyrazinamide	2000-2500mg

Table 2: Standardised National MDR-TB Treatment Regimen for Adults and Adolescents (Continuation phase) [DOH, 2011].

This is taken at least 6 times a week, for at least 18 months after TB Culture Conversion:

Patient Weight:	Drug:	Dose:
<33kg	Moxifloxacin	400mg
	Ethionamide	15-20mg/kg
	Terizidone	15-20mg/kg
	Pyrazinamide	30-40mg/kg
33-50kg	Moxifloxacin	400mg
	Ethionamide	500mg
	Terizidone	750mg
	Pyrazinamide	1000-1750mg
51-70kg	Moxifloxacin	400mg

	Ethionamide	750mg
	Terizidone	750mg
	Pyrazinamide	1750-2000mg
>70kg	Moxifloxacin	400mg
	Ethionamide	750-1000mg
	Terizidone	750-1000mg
	Pyrazinamide	2000-2500mg

The following drugs are used in the treatment of drug-resistant TB in South Africa [DOH, 2011]: amikacin/kanamycin, PZA, ethambutol, capreomycin, ethionamide, moxifloxacin/ofloxacin/levofloxacin, cycloserine/terizidone and para-aminosalicylic acid (PAS).

Other drugs that may be used in the treatment of drug-resistant TB are thioacetazone, clofazimine, amoxycillin-clavulanate, clarithromycin, azithromycin, rifabutin, rifapentine, high-dose isoniazid, imipenem and linezolid [DOH, 2011].

The diagnosis of DR-TB is also costly and more complicated [Friedland, 2007_a]. Treatment must be individualized and tailored according to the susceptibility of the patient's isolate. Resources are needed to provide adherence counseling, psychosocial support and management of side

effects. Second-line drugs are expensive and not always easy to source, and their quality may be questionable [MSF, 2011].

Research into the development of new anti-TB drugs has largely been neglected as the disease mainly affects developing countries and is therefore not a very lucrative market for pharmaceutical companies. Drugs used for treating MDR-TB have limited efficacy with success rates around 80%, at best, and the international organization MSF feels that in the absence of newer, better therapies, there is an urgent need to improve existing regimens used for MDR-TB; to maximize their effectiveness while making them more tolerable and minimizing their side effects [MSF, 2011].

Side effects of anti-TB drugs may include; nausea, vomiting, diarrhea, vertigo, hearing loss, joint pain, peripheral neuropathy, hepatotoxicity, depression and hallucinations [DOH, 2009_b; MSF, 2011]. In a study done in Nepal [Koju et al, 2005], which looked at the occurrence of side effects of anti-TB drugs, it was reported that 80% of patients experienced at least one type of side effect, with 34.29% experiencing major side effects. Female gender, history or alcohol abuse and positive sputum smear were factors associated with an increased risk for occurrence of these side effects. Pande et al [1996] looked at the risk factors associated with hepatotoxicity in patients taking antimycobacterial drugs for the treatment of TB. They found that increased alcohol intake, hypoalbuminaemia, age and advanced disease were all factors associated with an increased incidence of hepatitis.

2.3 Pharmacology of anti-tuberculosis drugs:

2.3.1 Rifampicin

Rifampicin is bactericidal for intra- and extra cellular bacteria and inhibits bacterial RNA synthesis by interfering with DNA-dependant polymerase [Chambers, 2004]. The oral bioavailability is reduced by food and by first-pass metabolism. It is well distributed, with cerebrospinal fluid (CSF) concentrations reaching 10-20% of the plasma concentrations. Protein binding is 80-90% and rifampicin has a half-life of 2.5hrs (which is increased in hepatic disease). It is metabolized rapidly in the liver and undergoes autoinduction. Rifampicin and its active deacetylated metabolite are eliminated chiefly via the biliary-faecal route and 60-65% is excreted unchanged in the faeces, the remainder in the urine [Rossiter, 2010].

Adverse effects of rifampicin include hepatotoxicity (which is increased in the prescence of other hepatotoxic drugs like isoniazid), gastro-intestinal disturbances such as nausea, vomiting and diarrhoea, central nervous system (CNS) disturbances and hypersensitivity reactions. The drug may cause a red-orange discolouration of body fluids such as urine and tears [Chambers, 2004]. Rifampicin should be used with caution in patients with pre-existing hepatic dysfunction and porphyria, and in patients taking other hepatotoxic drugs or alcohol. Rifampicin may interact with many drugs due to the fact that it is a potent inducer of certain enzymes in both the liver and intestine. The levels of these drugs may be decreased and may drop below therapeutic levels, requiring dosage adjustment Drugs

involved include: protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), phenytoin, warfarin, oral contraceptives, and digoxin. [Rossiter, 2010].

The usual adult dose is 10mg/kg orally (PO) or by intravenous injection (IVI) as a single daily dose [Chambers, 2004]. In patients with liver impairment, the total daily dose should not exceed 8mg/kg and in paediatrics the usual dose is 10mg/kg/day (although this may be increased to 20mg/kg/day in the treatment of miliary TB and TB meningitis [Rossiter, 2010].

2.3.2 Isoniazid

Isoniazid is bactericidal for intra- and extracellular mycobacteria and exerts its action by inhibiting mycobacterial cell wall synthesis [Chambers, 2004]. As well as being used in the treatment of TB, it is used prophylactically in IPT (Isoniazid Preventative Therapy). Oral absorption of isoniazid is good, although this is decreased when taken simultaneously with food or antacids, and by first-pass metabolism [Rossiter, 2010]. It is distributed widely, including in the CNS and has a half-life of 1-6 hours. It undergoes metabolism in the liver, mainly by acetylation and some unchanged isoniazid and inactive metabolites are excreted in the urine [Chambers, 2004].

Isoniazid is hepatotoxic and may cause transient elevation of liver enzymes. It is also neurotoxic and may cause peripheral neuropathy. Because of this, pyridoxine is usually

given in conjunction with isoniazid therapy. Skin rashes, gastrointestinal disturbances and heamatological abnormalities have also been known to occur [Chambers, 2004].

Due its possible hepatotoxicity, caution should be exercised in patients with pre-existing liver impairment or porphyria, and in patients taking other potentially hepatotoxic drugs or alcohol. Drug interactions are common with isoniazid use due to the fact that isoniazid is an inhibitor of certain cytochrome P450 enzymes. Isoniazid will inhibit the metabolism of these drugs and hence their doses may need to be reduced in order to prevent toxicity. Drugs involved include: phenytoin, carbamazepine, warfarin and theophylline. The concomitant use of isoniazid with rifampicin, paracetamol or alcohol may potentiate hepatotoxic effects. In adults, isoniazid is dosed at 5mg/kg daily as a single oral dose and in paediatrics between 5 and 10mg/kg/day. In TBM (TB meningitis) and miliary TB, 20mg/kg/day may be given. When INH is given as prophylaxis (IPT), the dose is 10mg/kg daily (maximum 300mg/day) [Rossiter, 2010].

2.3.3 Ethambutol

Ethambutol is bacteriostatic, but may be bactericidal at higher doses. It is hypothesized that it exerts its effect by inhibiting ribonucleic acid (RNA) synthesis in actively dividing mycobacteria [Rossiter, 2010]. It is used in the treatment of DS-TB and DR-TB, and has low toxicity and good tolerance [DOH, 2011]. Ethambutol is well distributed except in the CSF. It has an elimination half-life of 3-4 hours in healthy patients, which is increased in patients with renal impairment. Approximately 15% is metabolized in the

liver, and it is eliminated chiefly by the excretion of the unchanged drug in the urine. The most common side effects experienced are optic neuritis and hyperuricaemia (which may cause arthralgia and gout). Other less common side effects are gastrointestinal disturbances, peripheral neuropathy and skin rashes. The use of ethambutol is contra-indicated in patients with advanced renal failure and in those with optic neuritis. It should be used with caution in patients with renal impairment and eye defects. Ethambutol is not recommended for use in children younger than 8 years old except in severe cases where the benefit outweighs the risk. Ethambutol interacts with PZA and thiazide diuretics causing an increased risk of elevated serum urate levels. When used with other neurotoxic drugs, the risk of optic and peripheral neuritis is increased [Rossiter, 2010].

In adults and paediatrics, ethambutol is given at a dose of 15mg/kg orally once daily, with doses up to 25mg/kg being used for TB meningitis [Chambers, 2004]. In geriatric patients, the lowest dosing range should be used (maximum 15mg/kg daily) and in patients with renal impairment the dosage intervals should be increased [Rossiter, 2010].

2.3.4 Streptomycin/Amikacin/Kanamycin

Streptomycin was the first antimycobacterial drug to be discovered. Aminoglycosides exert their antimicrobial action by inhibiting bacterial protein synthesis by binding to the 30S ribosomal subunit and are bactericidal against actively dividing bacteria [DOH, 2011].

Following intramuscular (IM) injection, peak concentrations of aminoglycosides in the plasma are reached in approximately 30 – 90 minutes. This class of drugs exhibits concentration-dependant killing and a significant post-antibiotic effect; therefore it is recommended that they be given as a single daily dose [Chambers, 2004]. Aminoglycosides are cleared via the kidneys and have a half-life of approximately 2-3 hours in patients with normal renal function. Therapeutic drug monitoring is useful in patients with renal impairment so as to avoid or minimise accumulation and toxicity, dosing intervals may need to be increased depending on plasma levels dose [Rossiter, 2010.

The most common and serious side effects of aminoglycosides are nephrotoxicity and ototoxicity [Chambers, 2004]. Nephrotoxicity is more likely in patients with renal impairment or those taking other nephrotoxic agents concurrently. The use of aminoglycosides with other nephrotoxic or ototoxic drugs is not recommended due to the additive potential for toxic effects. When used with neuromuscular blocking agents, blockade may be potentiated. Hypersensitivity reactions and CNS effects such as headaches may also occur. Aminoglycosides are contra-indicated in patients who are hypersensitive to them and they are not recommended for use in pregnancy as they cross the placenta and may cause fetal ototoxicity. They should be used in caution in patients with renal impairment, hearing impairment and myasthenia gravis [Rossiter, 2010].

The usual adult dose for aminoglycosides is 15mg/kg. In patients with renal impairment, the dosing interval should be prolonged and the dose reduced according to plasma levels.

Streptomycin is given by IM injection only, amikacin and kanamycin may be given IM or IV [Rossiter, 2010].

2.3.5 Capreomycin

Capreomycin is a cyclic polypeptide that inhibits protein synthesis in mycobacteria. It is given by intramuscular injection. A daily dose of 1g yields plasma concentrations of approximately 10mcg/ml [Chambers, 2004]. Capreomycin is classed as having moderate tolerance and medium toxicity, and its use is commonly limited by its nephrotoxicity) [DOH, 2011]. It is also frequently ototoxic, and may also cause hypersensitivity reactions such as a skin rash. Capreomycin should be used with caution in patients with renal or hearing impairment, and in patients taking aminoglycosides [DOH, 2011].

The adult dose for capreomycin is 15mg/kg daily IMI (maximum 20mg/kg/day) [DOH, 2011]. In patients older than 50 years, this is decreased to10mg/kg/day (maximum 750mg/day). Paediatric patients are dosed at 15-30mg/kg/day (maximum 1g/day), in patients with renal impairment the dose should be decreased, and the dosing interval should be increased [Chambers, 2004].

2.3.6 Ethionamide

Ethionamide is bacteriostatic, but bactericidal at higher concentrations [DOH, 2011]. It is a synthetic thiocarbamide derivative of isonicotinic acid. Ethionamide is widely distributed, including into the CNS. It has a half-life of 3 hours and is metabolized mainly in the liver. Active and inactive metabolites and approximately 1% of the unchanged drug is excreted into the urine [Rossiter, 2010].

Gastrointestinal disturbances are the most common, particularly nausea, vomiting and metallic taste. This may be alleviated somewhat by dividing the total daily dose. CNS effects such as psychosis, depression and anxiety may also occur. The use of ethionamide is contra-indicated in patients hypersensitive to the drug, as well as patients with severe hepatic dysfunction and porphyria. Caution should be exercised when using ethionamide in patients with a history of psychiatric illness, epilepsy, diabetes or hypothyroidism [Rossiter, 2010].

When ethionamide is used with isoniazid, there is an increased risk of neurological side effects. When it is used with cycloserine or terizidone, there is an increased risk of CNS side effects. In adults and paediatrics older than 10 years, the usual dose is 15-20mg/kg daily as a single oral dose (maximum 1g/day). Patients with renal impairment with a glomerular filtration rate (GFR) less than 10ml/min should be given 50% of the normal dose. Paediatrics less than 10 years are given 10-15mg/kg daily (this may be increased to 20mg/kg daily in miliary TB or TB meningitis)[Rossiter, 2010].

2.3.7 Moxifloxacin/Ofloxacin/Levofloxacin

Fluoroquinolones are bactericidal and exert their effect by inhibiting deoxyribonucleic acid (DNA) gyrase and therefore bacterial DNA production [Chambers, 2004]. Moxifloxacin is preferred in the treatment of MDR and XDR-TB but ofloxacin and levofloxacin are preferred in patients younger than 8 years old [DOH, 2011].

Fluoroquinolones are well absorbed following oral administration and distributed widely in the tissues. They are excreted renally. The half-life of ofloxacin and levofloxacin is 5-7 hours while the half-life of moxifloxacin is 9-10 hours [Chambers, 2004]. The fluoroquinolones are generally well tolerated with common side effects being gastrointestinal such as nausea and diarrhoea. Quinolones are contraindicated in patients who have a known allergy or sensitivity to them. Caution should be exercised when using them in patients with hepatic or renal impairment or in patients under 18 years old as they may damage growing cartilage. They are not recommended for use in pregnant or lactating women [Rossiter, 2010]. Fluoroquinolones should not be taken with antacids or minerals such as calcium, iron or magnesium as they may complex with the drug and reduce absorption [Chambers, 2004]. Levofloxacin is given to adults in a dose of 7.5-10mg/kg daily (maximum 1000mg/day) and to paediatric patients younger than 5 years old in a dose of 10mg/kg twice daily. Paediatric patients older than 5 years are given 10mg/kg once daily. The adult dose for moxifloxacin is 400mg daily (orally or IV), and the dose for children is 7.5mg-10mg/kg daily. Ofloxacin is dosed at 800mg daily for adults (oral or IV) [DOH, 2011].

2.3.8 Terizidone/Cycloserine

Terizidone is a cycloserine derivative but has a lower incidence of side effects than cycloserine. It is a combination of 2 molecules of cycloserine and therefore is widely regarded as the same drug effects [DOH, 2011]. It is bacteriostatic and inhibits mycobacterial cell wall synthesis by interfering with peptidoglycan pentapeptide production [Chambers, 2004]. Terizidone shows good absorption and distribution into tissues and fluids including the CSF [Rossiter, 2010]. It is metabolized by the kidneys and the majority of a dose is excreted in active form into the urine. Terizidone commonly causes a range of neurological side effects such as seizures, psychosis, depression, anxiety, drowsiness, tremor and dizziness. These are most common in patients with renal impairment or those receiving high doses [Chambers, 2004]. High dose pyridoxine (150mg) should be given concomitantly to minimize neurological side effects [DOH, 2011]. Terizidone use is contra-indicated in patients with porphyria, psychosis, depression, epilepsy and severe renal impairment. It is given to adults at a dose of 10-20mg/kg daily orally (maximum 750mg/day). The dosing interval should be prolonged for patients with renal impairment [Rossiter), 2010].

2.3.9 Para-aminosalicylic acid (PAS)

PAS is bacteriostatic and acts by inhibiting mycobacterial folate synthesis. PAS is readily absorbed after oral administration and is distributed widely in tissues and fluids except

the CSF. It has a half-life of about 1 hour and is excreted in the urine both as the unchanged drug and its metabolites [Chambers, 2004].

It frequently causes gastrointestinal disturbances such as anorexia, nausea and diarrhoea, but is generally classed as having low toxicity. It may also cause hepatitis, hypothyroidism, goitre and a malabsorption syndrome [DOH, 2011].

PAS is contra-indicated in patients with severe renal insufficiency. PAS inhibits the absorption of rifampicin and digoxin from the gastro-intestinal tract and therefore the administration times of these drugs should be separated when given concurrently. The adult dose for adults is 8-12g daily po in 2 divided doses, and for paediatrics: 150-300mg/kg/day in 2-4 divided doses (maximum 10g/day). The use of PAS is limited by its acceptability, as it is bulky and unpleasant to take [DOH, 2011].

2.3.10 Pyrazinamide

PZA is bactericidal in an acidic environment (such as that found in bacterial macrophages) [Zhang et al 2003]. Many MDR-TB patients have chronically inflamed lungs, which also theoretically provide an acidic environment. It inhibits mycobacteria at concentrations of approximately 20mcg/ml [Chambers, 2004]. It has a sterilizing effect and is active against dormant bacteria, thereby allowing the treatment period to be shortened and playing a crucial role in the treatment of MTB.

PZA is a nicotinamide analogue with the molecular formula $C_5H_5N_3O$, its structure is shown in Figure 1 below:

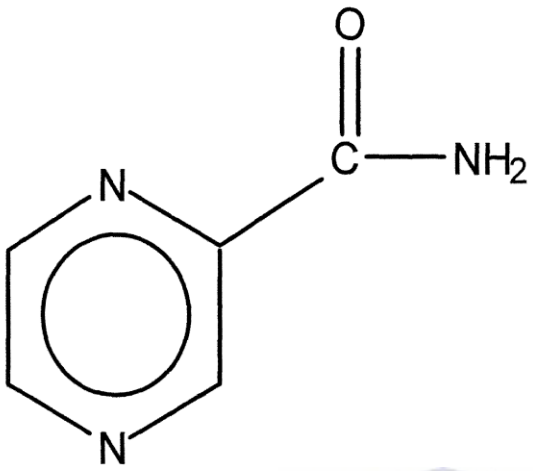

Figure 1: Molecular structure of pyrazinamide [Zhang and Mitchison, 2003]

The mode of action of PZA is poorly understood, although it is proposed that the drug is deaminated by PZA-susceptible strains of MTB into the active metabolite pyrazinoic acid, and that this metabolite disrupts membrane energetics and inhibits membrane transport function in MTB. [Salfinger and Heifets, 1988; Zhang et al, 2003]. Early research by Salfinger and Heifets [1988] shows that for PZA, the minimum inhibitory concentration (MIC) is dependent upon pH. The MIC for MTB at pH 5.5 is 50mcg/ml, whereas at pH 5.95, the MIC is around 400mcg/ml.

PZA is only used in the treatment of mycobacterial infections and is used in combination with other anti-TB drugs. PZA is well absorbed from the gastrointestinal tract and widely distributed, reaching concentrations in the CSF equal to those in the plasma. It has a half-life of 9-10 hours, and 4-14% of the drug is eliminated unchanged in the urine, the remainder as metabolites formed in the liver [Rossiter, 2010].

The most important side effect noted is dose-related hepatotoxicity, as well as GIT side effects such as nausea, vomiting and diarrhoea, although these may be minimized if the drug is taken with meals. It may also cause hyperuricaemia (due to PZA inhibiting uric acid clearance) and this may be associated with non-gouty arthralgia. Liver function tests and serum uric acid levels should be regularly monitored. Photosensitivity, thrombocytopaenia and sideroblastic anaemia are rare. PZA is contraindicated in patients with porphyria, or those with severe hepatic damage, and should be used in caution in patients with gout, diabetes, renal impairment, and in patients who exhibit hypersensitivity to PZA, isoniazid, ethionamide or niacin. PZA inhibits urate clearance, and therefore allopurinol and probenicid dosages may need to be adjusted if used concurrently. When PZA is used with diuretics and ethambutol, there is an increased additive potential for serum urate levels to be elevated [Rossiter, 2010].

The adult dose of PZA is 20-30mg/kg/day (maximum of 2g/day) [DOH, 2011]. For patients with renal impairment, the dose at the lower limit of the recommended range should be used, although if the patient's GFR is less than 10ml/min the dose may be reduced by up to 50%. In paediatric patients the dose used is 15-30mg/kg daily (up to 40mg/kg/day in TB meningitis, miliary and MDR-TB) [Rossiter, 2010].

When PZA's pharmacokinetics was studied under fasting conditions, with food, and with antacids, the T_{max} was mostly around the 1-hour mark, indicating rapid absorption. The researchers discovered that the presence of food and antacids did not substantially affect the pharmacokinetics of PZA [Peloquin et al, 1998]. Tappero et al [2005] also reported good absorption of PZA in both HIV positive and HIV negative populations.

Researchers in Canada studied the incidence of serious side effects of first-line anti-TB drugs in patients being treated for TB and found that PZA caused a substantially higher incidence rate of side effects like hepatotoxicity and rash than the other 3 drugs [Yee et al, 2003].

2.3.10.1 Methods used in the determination of Pyrazinamide levels in plasma

According to Peloquin [2002], the preferred methods used in the assay of antimycobacterial drugs are high-performance liquid chromatography (HPLC) and gas chromatography (GC) with UV detection or mass spectrometry.

Most previous studies have either used HPLC with UV detection, or LC-MS to determine the levels of PZA in plasma.

Unsalan et al [2006] used HPLC to assay the plasma levels of PZA, INH and rifampicin in 25 patients. They found the method to be simple, accurate, precise and reproducible, with the lower limit of quantification for PZA at 1.5mg/L and noted that many of the

levels were found to be subtherapeutic. Revankar et al [2013] also used HPLC to determine PZA levels in human plasma. They found it sensitive and selective.

Chaitanya Krishna et al [2012] outlined their method for determining PZA levels in individual dosage forms and fixed-dose combinations. They used reverse phase liquid chromatography to separate the compounds, and a triple quadrupole mass spectrometer with electron spray ionization in the positive mode for ion detection. They describe this method as simple and selective.

Shah et al [2012] used a similar method; also using reverse-phase liquid chromatography and a triple quadrupole mass spectrometer with electrospray ionization in the positive mode. They validated this method over a wide range of concentrations and found it to be sensitive and selective.

Gong et al [2009], developed and validated a method of simultaneously analyzing the levels of PZA and ethambutol in human plasma using LC-MS. Their method utilized a HPLC system coupled with a triple quadrupole mass spectrometer using an atmospheric pressure chemical ionization source and operating in positive mode. They found their method to be rapid and robust, with a short run-time. Zhou et al [2013] also successfully used a LC-MS with a HILIC (hydrophilic interaction liquid chromatography) column to quantify PZA levels in a TDM study.

2.3.10.2 Pyrazinamide pharmacokinetic data from the literature

Table 3: Population pharmacokinetic parameters for pyrazinamide in healthy subjects-mean (SD):

Population:	**Dose:**	**C_{max} (mcg/ml):**	**T_{max} (hr):**	**$T_{1/2}$ (hr):**	**AUC (mg.hr/L):**	**Analysis:**	**Reference:**
9 Healthy adults	27mg/kg	38.7(5.9)	1	9.6(1.8)	520(101)	HPLC with fluorimetry	Lacroix et al, 1989
24 Healthy adult males	1,5g	29.21(4.35)	1,17(0.41)	9.58	415.46	HPLC-UV	Peloquin et al, 1997
14 Healthy adults (fasting)	30mg/kg	53.4	1.43		673	GCMS	Peloquin et al, 1998
14 Healthy adults (fasting)	30mg/kg	52.1	1.71		680	GCMS	Peloquin et al, 1998
14 Healthy adults (after high-fat meal)	30mg/kg	45.6	3.09		687	GCMS	Peloquin et al, 1998
14 Healthy adults (with antacid)	30mg/kg	55.7	1.43		628	GCMS	Peloquin et al, 1998

Table 4: Population pharmacokinetic parameters for pyrazinamide in TB patients-mean (SD):

Population:	**Dose:**	**C_{max} (mcg/ml):**	**T_{max} (hr):**	**$T_{1/2}$ (hr):**	**AUC (mg.hr/L):**	**Analysis:**	**Reference:**
18 HIV+ children	22-44mg/kg 3 x weekly	34.0 (18.1)	3.7 (1.7)	-	411 (382)	HPLC-UV	Graham et al, 2006
9 HIV– children	22-44mg/kg 3 x weekly	41.9 (22.9)	3.7 (1.7)	-	322 (240)	HPLC-UV	Graham et al, 2006
15 Children 0-4 years	22-44mg/kg	27.5 (16.6)	3.5 (1.6)	-	327 (335)	HPLC-UV	Graham et al, 2006
12 Children 5 or over	22-44mg/kg 3 x weekly	47.9 (17.7)	3.3 (1.4)	-	416 (333)	HPLC-UV	Graham et al, 2006
6 Children >80% WFA	22-44mg/kg 3 x weekly	44.3 (17.1)	3.7 (1.8)	-	496 (407)	HPLC-UV	Graham et al, 2006
12 Children 60-80% WFA	22-44mg/kg 3 x weekly	33.7 (23.2)	3.4 (1.4)	-	312 (261)	HPLC-UV	Graham et al, 2006
9 Children < 60% WFA	22-44mg/kg 3 x weekly	35.4 (16.8)	3.2 (1.6)	-	337 (337)	HPLC-UV	Graham et al, 2006
47 HIV+ adults	2g daily	42.17(11.29)	2.31(0.98)	9.05(4.12)	290.76	HPLC	Perlman et al, 2004
24 HIV+ adults	2.5g intermittent	52.81(17.58)	2.73(1.51)	9.04(3.94)	366.64(107.25)	HPLC	Perlman et al, 2004

Contd…

Population:	Dose:	C_{max} (mcg/ml):	T_{max} (hr):	$T_{1/2}$ (hr):	AUC (mg.hr/L):	Analysis:	Reference:
40 Children	30mg/kg/day	43.43(6.74)	2.25(0.77)	7.78(1.3)	496.11(138.15)	HPLC-UV	Arya et al, 2006
71 HIV+ adults CD4>200	1,5-2g	49.9	-	-	-	GC-MS	Chideya et al, 2009
84 HIV+ adults CD4<200	1.5-2g	46.9	-	-	-	GC-MS	Chideya et al, 2009
70 HIV- adults	1.5-2g	52.3	-	-	-	GC-MS	Chideya et al, 2009
141 Mostly HIV- adults	35.7mg/kg/day	52,7	2	5,9	499,7	HPLC-UV	McIlleron et al, 2006
18 Adults with Type 2 diabetes	32,5	45,5	1		409	HPLC	Ruslami et al, 2009
18 Adults without Type 2 diabetes	32,2	47	1,5		468(	HPLC	Ruslami et al, 2009

Contd…

Population:	Dose:	C_{max} (mcg/ml):	T_{max} (hr):	$T_{1/2}$ (hr):	AUC (mg.hr/L):	Analysis:	Reference:
11 Children with TB	25mg/kg	42.4 (3.3)	1.7 (0.2)	9.3 (1.3)	561 (99)	UV-spec	Gupta et al, 2007
11 Children with TB	15mg/kg	38.6 (3.9)	1.8 (0.1)	10.5 (2.3)	515 (89)	UV-spec	Gupta et al, 2007
7 Children with TB	31.9mg/kg	49.4 (2.8)	2	7.8 (1.1)	435.0 (44.2)	UV-spec	Roy et al, 2012
13 Children with TB	28.1mg/kg	41.7 (1.2)	2	6.6 (0.6)	316.6 (20.7)	UV-spec	Roy et al, 2012
14 HIV+ Adults with TB	1,5g	32.1 (8.8)	-	7.4 (1.6)	350 (111)	HPLC	Choudhri et al, 1997
Contd…							
15 HIV- Adults with TB	1,5g	33.1 (8.2)	-	6.9 (2.1)	382 (143)	HPLC	Choudhri et al, 1997
11 Adults with TB and diarrhoea	1,5g	33.9 (9.0)	-	7.8 (1.9)	407 (148)	HPLC	Choudhri et al, 1997
18 Adults with TB without diarrhea	1,5g	31.7 (8.1)	-	6.7 (1.8)	336 (103)	HPLC	Choudhri et al, 1997

Contd…

Population:	Dose:	C_{max} (mcg/ml):	T_{max} (hr):	$T_{1/2}$ (hr):	AUC (mg.hr/L):	Analysis:	Reference:
13 Adults with TB and AIDS	29.9mg/kg	55.9	1.33	-	373.2	HPLC	Taylor and Smith, 1998
14 Adults with TB without AIDS	29.9mg/kg	56.9	1.36	-	385.3	HPLC	Taylor and Smith, 1998
59 Adults with TB with HIV	1,5-2g	48.7	2	9.19	385	GCMS	Tappero et al, 2005
28 Adults with TB without HIV	1,5-2g	55.5	2	7.41	424	GCMS	Tappero et al, 2005

2.4 HIV/AIDS:

2.4.1 Introduction:

Human Immunodeficiency Virus is a type of retrovirus, which infects cells in the host's immune system, and destroys them or impairs their ability to fight infection. As a result of this, the host's immune system may become incapable of fighting off infections and the patient may succumb to an illness that he or she would normally be able to fight off. Such infections are called "Opportunistic Infections". HIV infection causes the syndrome known as AIDS (or Acquired Immune Deficiency Syndrome). The virus may be transmitted in the following ways; through unprotected sexual intercourse (anal or vaginal) through the transfusion of contaminated blood, through the sharing of contaminated needles or between a mother and her infant during pregnancy, childbirth or breastfeeding [WHO, 2013_d].

2.4.2 Common opportunistic infections:

As a patient's CD4 count decreases, he/she will become more and more susceptible to infections. The conditions that most often affect HIV positive patients in South Africa include TB (pulmonary and extra-pulmonary), sepsis, pneumonia, oral and oesophageal candidiasis, herpes Zoster, Kaposi's sarcoma, crytococcal meningitis, pneumocystis pneumonia, toxoplasmosis and cytomegalovirus [Wilson et al, 2008].

2.4.3 Effects of HIV infection on the GIT:

Most HIV positive patients experience gastro-intestinal tract (GIT) symptoms or disease at some stage. These include odynophagia or dysphagia, oesophageal candidiasis, apthous ulcers or herpes simplex ulcers and diarrhoea. Diarrhoea in HIV positive patients is common, and many patients will present with diarrheoa at some stage in their illness. Common causative organisms are Coccidia (*Cryptosporidia, Microsporidia, Isospora belli and Cyclospora),* bacteria *(Salmonella, Shigella, Campylobacter and Clostridium difficile),* mycobacteria *(TB or MAC)* and viruses *(cytomegalovirus or HIV).* Diarrhoea may also be caused by some antiretrovirals (particularly with Lopinivir/Ritonivir), antiTB drugs, or other drugs used to treat opportunistic infections. It may also be due to HIV associated enteropathy, which is associated with malabsorption [Wilson et al, 2008].

2.4.4 Hepatic HIV manifestations:

Hepatic disease in HIV positive patients may be caused by hepatic viruses, the HI virus itself, opportunistic disease or medication. Antiretrovirals such as the NNRTI's and protease inhibitors and anti-TB drugs such as PZA, isoniazid and rifampicin are associated with hepatotoxicity [DOH, 2009]. Co-infection with HIV and hepatitis B is common, and these patients are at an increased risk for chronic infection and chronic liver disease. They also are also more susceptible to drug-induced hepatotoxicity and may develop hepatitis B associated immune reconstitution inflammatory syndrome (IRIS) [Wilson et al, 2008:413-414]. Patients with hepatic disorders

may require dose adjustment of drugs including some ARV's and antimycobacterials [Wilson et al, 2008].

2.4.5 Renal complications of HIV/AIDS:

HIV infection is associated with both acute and chronic renal disease. The involvement of the virus on the kidneys may be direct or indirect and can result in acute tubular necrosis, acute interstitial nephritis, TB of the urological tract (kidneys, ureters, bladder), crystalluria (which may be associated with use of protease inhibitors), HIV-associated nephropathy ("HIVAN"), HIV immune complex kidney disease as well as other glomerulonephritidies.

The dose of some of the nucleoside reverse transcriptase inhibitors may need to be decreased in patients with impaired renal function. Tenofovir commonly causes impaired renal function. Streptomycin and ethambutol are excreted by the kidney, and may need to be given in reduced doses [Wilson et al, 2008]. Patients with renal disorders may require dose adjustment of drugs including some antiretrovirals (ARVs) and antimycobacterials [Wilson et al, 2008].

2.4.6 Treatment of HIV/AIDS:

HIV/AIDS is treated with antiretrovirals. The 3 classes used in South Africa are Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs). NRTIs prevent the conversion of viral RNA into

proviral DNA, while NNRTIs cause a conformational change in the enzyme reverse transcriptase, thereby inhibiting it. Protease inhibitors work by interfering with the function of the enzyme protease which cleaves viral polyproteins into functional proteins [Wilson et al, 2008].

The regimens used for the treatment of HIV/AIDS in South Africa are summarised below:

Table 5: Standardised National ART regimens for adults and adolescents [DOH, 2013]:

Patients:	**Regimen:**
All new patients needing treatment, including pregnant women	Tenofovir (TDF) + Emtricitabine(FTC) or Lamivudine (3TC) + Efavirenz (EFV)
Patients with significant psychiatric co-morbidity or where EFV might impair daily functioning (shift workers) or patients in whom EFV use is contraindicated	TDF + FTC/3TC + Nevirapine (NVP)
Adolescents	Abacavir (ABC) + 3TC + EFV
Contraindication to TDF (renal disease or concurrent	Zidovudine (AZT) + 3TC +

use of other nephrotoxic drugs)	EFV/NVP
Contraindication to TDF and AZT (anaemia and renal disease or concurrent use of other nephrotoxic drugs)	Stavudine (d4T) + 3TC + EFV/NVP
Contraindication to TDF and AZT and d4T (anaemia and peripheral neuropathy and renal disease or concurrent use of other nephrotoxic drugs)	ABC + 3TC + EFV/NVP
Patients currently on d4T-based regimen	TDF + FTC/3TC + EFV

Common side effects of ARV therapy include lactic acidosis (particularly stavudine), hepatotoxicity (particularly nevirapine), lypodystrophy/lipoatrophy (particularly long-term use of stavudine or protease inhibitors), psychiatric symptoms and hallucinations (efavirenz), gynaecomastia, peripheral neuropathy and rash that may progress to Steven-Johnson's Syndrome in serious cases [Wilson et al, 2008].

2.5 Tuberculosis and HIV/AIDS: A lethal combination:

TB is the most common opportunistic infection that infects patients with HIV/AIDS and is a leading cause of death in these patients. The combination of TB and HIV/AIDS is lethal, and the diseases have common biologic, environmental and social etiologies. TB is the leading cause of morbidity and mortality in patients with HIV/AIDS, and is responsible for 1 in every 4 AIDS-related deaths [WHO, 2013_c]. The WHO suggests collaboration and integration between TB and HIV/AIDS control programs. This could improve diagnosis, treatment and outcome for patients co-infected with both diseases. Such programs require collaboration of policies at a national level and resources such as adequate staff, financial support, equipment and medication. [Friedland et al, 2007_b:S121; WHO, 2012_b].

Treatment of patients co-infected with TB and HIV is complex. Not only does it involve patients having to take large amounts of medication, but also it is further complicated by three major issues: drug interactions, overlapping toxicities and immune reconstitution inflammatory syndrome [McIlleron et al, 2007].

Firstly, there may be interactions between antiretroviral and antimycobacterial drugs, and their effects on the body. Rifampicin causes the induction of CYP450 and p-glycoprotein [Wilson et al, 2008], this causes decreased levels of rifampicin (auto-induction), and other drug interactions (particularly with protease inhibitors and NNRTI's) involving the CYP3A enzyme system [Coyne et al, 2009].

Secondly, there is the issue of overlapping toxicities, with many of the drugs used to treat both conditions having similar side effect profiles. Skin rash, nausea, vomiting and hepatitis are

associated with all four first-line anti-TB agents as well as some of the antivirals (e.g.: efavirenz, nevirapine and some protease inhibitors) [McIlleron et al, 2007; Burman and Jones, 2001]. There is an increased risk of peripheral neuropathy with concomitant use of ethambutol, ethionamide or isoniazid with stavudine or didanosine, these patients should be monitored carefully and appropriate prophylaxis or treatment given simultaneously [Swart and Jones, 2009]

Lastly, there is the issue of immune reconstitution inflammatory syndrome (IRIS), where a previously suppressed immune system is suddenly activated and may cause a paradoxical clinical deterioration. Immune reconstitution can cause a sudden worsening of symptoms in severely immunocompromised patients starting on ARV therapy, as the patient's immune system's start to recover and mount a response to the opportunistic infection [McIlleron et al, 2007; Burman and Jones, 2001]. Ideally, ARV initiation should be deferred until the patient is tolerating TB therapy (2-4 weeks). The patient should be carefully monitored during this time [DOH, 2013].

Although there is limited evidence to suggest this, zidovudine may decrease levels of PZA [Swart and Jones, 2009]. Rifampicin is a potent CYP450 isoenzyme inhibitor and as such may decrease the plasma concentrations of abacavir, efavirenz, nevirapine, zidovudine and lopinivir/ritonavir. Where rifampicin is used with lopinivir/ritonavir, the lopinivir/ritonavir dose should either be doubled or extra ritonavir should be added [Swart and Jones (eds), 2009]. Streptomycin and tenofovir are both nephrotoxic agents and thus this combination should be avoided [Swart and Jones, 2009].

2.6 Pharmacokinetics of the antimycobacterial drugs in HIV positive and HIV negative patients:

Several authors have shown concern that patients with HIV may exhibit impaired absorption of antiTB drugs due to the possibility of malabsorption associated with retroviral disease [Graham et al, 2006; McIlleron et al, 2006; Gurumurthy et al, 2004_a: 282 and 2004_b]. This could possibly lead to sub-therapeutic levels, i.e. below the minimum inhibitory concentration and hence the spread of resistance.

Berning et al [1992], report the case of a HIV-positive health care worker with suspected MDR-TB. Low plasma concentrations as well as stool analysis indicated a malabsorption disorder. Patel et al [1995] report 2 HIV positive patients who suffered relapse with drug-resistant TB. These patients were evaluated for malabsorption, and while one of the patients showed no evidence of this, the other patient had chronic diarrheoa and an abnormal D-xylose absorption test. It is suspected that this may have been due to HIV enteropathy and that this may have contributed to subtherapeutic drug levels and consequently the development of drug-resistant TB in this patient.

Evidence has been found of malabsorption of rifampicin and isoniazid in patients with HIV and diarrhoea and HIV and TB [Gurumurthy et al [2004_a]. They investigated the urinary excretion of rifampicin and INH and their metabolites and found this was lower in both groups of HIV patients than in healthy subjects and HIV-negative TB patients. They also looked at the effect of HIV on the blood and urine levels of the first-line drugs. They found that HIV positive patients and HIV positive patients with TB, who had a history of diarrhoea and cryptosporidial infection,

exhibited decreased amounts of PZA and ethambutol excreted in the urine. They concluded that these patients had evidence of malabsorption and decreased bioavailability of these anti-TB drugs, and that this may be linked to poor treatment outcome [Gurumurthy et al, 2004_b].

A group of investigators in Canada [Sahai et al, 1997] also studied the pharmacokinetics of the 4 first-line drugs in HIV-positive patients who did not have TB. They discovered that the total systemic drug exposure was 32% lower for rifampicin and 24% lower for PZA in HIV-positive than in HIV-negative volunteers. They concluded that HIV positive patients, especially those in the advanced stages of the disease, showed decreased plasma concentrations of rifampicin and PZA. Pinheiro et al in Brazil [2006] studied the absorption of rifampicin and INH in patients with and without TB. Using urinary excretion of mannitol and lactulose as a measure of intestinal permeability, they found that almost all the patients had low levels of rifampicin or INH or both. They found however, that intestinal permeability was lower in the patients with TB than in those without TB, and suggest that intestinal absorption of these 2 drugs is decreased in patients with TB. Gupta et al [2007], reported no delayed absorption of PZA in children with TB, although there was wide interindividual variation of pharmacokinetics.

No association was found between AIDS or gender and concentrations of the antiTB drug ethionamide [Conte et al, 2000], and Li et al [2004], who studied the use of therapeutic drug monitoring (TDM) in MDR-TB patients, discovered that although HIV positive patients were more likely to have decreased concentrations of ethionamide and ofloxacin, these differences were not statistically significant.

Graham et al [2006] studied the pharmacokinetics of ethambutol and PZA in children with TB to examine the impact of age, nutritional status and HIV infection. They used HPLC with UV detection and found low serum levels of the drugs in all of the participants. They found that PZA C_{max} was lower in younger children (younger than 5 years) than in older, and lower in HIV infected children and those with severe malnutrition, although these differences did not reach statistical significance.

Low serum levels have also been reported in patients without HIV/AIDS. Bento et al describe a case of malabsorption of anti-TB drugs in a young HIV negative patient who failed to improve clinically on oral anti-TB drugs. Analysis of the patient's plasma showed very low or undetectable levels of the drugs, and hence malabsorption was suspected. The patient had no diarrhoea, normal serum albumin and investigations showed no GI abnormalities. The patient was switched to parenteral antimycobacterial therapy and began to improve clinically and radiographically, and was culture negative after three months.

Kimerling et al [1998], looked at the serum concentrations of the two most widely used anti-TB drugs; rifampicin and isoniazid, in HIV negative patients with TB. They found that 68% of patients showed low levels of INH and 64% had low levels of rifampicin. Although they did not find a statistically significant association between treatment failure and low serum antimycobacterial levels, they suggest that there may be a small population of patients for whom serum level monitoring might be appropriate and helpful, particularly in patients with drug-resistant strains or in patients who do not achieve culture conversion.

Low serum concentrations of the antimycobacterial drugs ciprofloxacin, ethambutol, rifampicin and clofazimine were also found in HIV-infected patients with *Mycobacterium avium* Complex. The investigators found concentrations well below the expected normal ranges and concluded that this was most likely due to malabsorption [Gordon et al, 1993].

It has also been found that MDR-TB patients had a significantly reduced intestinal absorptive area and significantly lower serum levels of rifampicin than patients with DS-TB and patients without TB [Barroso et al, 2009].

2.7 Aims of the study

The aim of this study was firstly to determine PZA plasma concentrations using LC-MS, secondly to describe the pharmacokinetic parameters of PZA in patients with MDR-TB and patients co-infected with MDR-TB and HIV and thirdly to find out if HIV infection influences the pharmacokinetics of PZA in patients with MDR-TB.

This was done in order to have a better understanding of the pharmacokinetics of this drug to see whether or not therapy could be optimized to provide effective antimicrobial cover while at the same time limiting dose-related side effects. This may help to improve patient outcomes and help prevent the spread of drug-resistant organisms.

2.8 Research questions:

- Can LC-MS be used to assay the plasma levels of PZA in patients with MDR-TB?
- What are the pharmacokinetic parameters for PZA in patients with HIV infection and in patients without MDR-TB infection?
- Is there a significant difference between the pharmacokinetics of PZA in MDR-TB patients with HIV infection and MDR-TB patients without HIV infection?

2.8.1 Experimental hypotheses:

2.8.1.1 LC-MS can be used to determine the plasma concentrations of pyrazinamide on patients with MDR-TB and patients with MDR-TB and HIV.

2.8.1.2 The difference between the pharmacokinetics of pyrazinamide in HIV positive patients with MDR-TB, and the pharmacokinetics of pyrazinamide in HIV negative patients with MDR-TB is statistically significant.

2.8.2 Null hypotheses:

2.8.2.1 LC-MS cannot be used to determine the plasma concentrations of pyrazinamide on patients with MDR-TB and patients with MDR-TB and HIV.

2.8.2.2 The difference between the pharmacokinetics of pyrazinamide in HIV positive patients with MDR-TB, and the pharmacokinetics of pyrazinamide in HIV negative patients with MDR-TB is not statistically significant.

2.8.3 General objectives:

The general objectives of this study were to determine the plasma concentrations of PZA in blood samples of MDR-TB patients using LC-MS, to describe the pharmacokinetics of PZA at the doses used for the treatment of MDR-TB in South Africa, and to assess whether or not HIV infection influences the pharmacokinetics of PZA

2.8.4 Specific Objectives:

The first specific objectives was to determine the plasma concentrations of PZA in patients with MDR-TB and in patients with MDR-TB and HIV/AIDS, at specific time periods after the administration of oral PZA, using LC-MS to analyse the drug levels in the plasma.

The second was to use these values in order to determine the following pharmacokinetic parameters for PZA:

* The maximum plasma concentration (C_{max})

* The time to reach the maximum plasma concentration (T_{max})

* The elimination rate constant (K_e)

* The half-life ($T_{1/2}$)

* The area under the plasma concentration vs. time curve from 0 to 24 hours (AUC_{0-24})

* The area under the plasma concentration vs. time curve from 0 to infinity ($AUC_{0-\infty}$)

* The volume of distribution (V_d)

* The clearance (Cl)

* The mean residence time (MRT)

* The absorption rate constant (K_a)

The third objective was to compare these pharmacokinetic parameters in both populations using statistical analysis to determine if there were significant differences between them.

CHAPTER 3:

RESEARCH METHODOLOGY

3.1. Study site, design, and population:

The study was conducted at the Brewelskloof Hospital (BKH) in Worcester, in the Western Cape province of South Africa. BKH is one of several South African hospitals specialising in the treatment of MDR-TB. The study was designed as a two-group, non-randomized pharmacokinetic study involving male and female HIV-positive (experimental group) and HIV-negative patients (control group) both admitted for MDR treatment.

3.2 Inclusion and exclusion criteria:

Inclusion criteria were; signature of informed written consent form after aims, procedures, advantages and disadvantages for the study had been clearly explained to the patient in their first language, consent for HIV test if not already done, on treatment for MDR-TB for at least two weeks and aged between 18 – 65 years.

Exclusion criteria were; patient request, history of any disease known to interfere with the pharmacokinetics of PZA, pregnancy, breast-feeding, intolerance to PZA, patients taking medicines known to interact with PZA and severe dehydration.

The following information was recorded on a specially designed data collection form: weight, age, TB history, current therapy, other (non-TB) history and concurrent drug therapy (including

ART), time of medication administration, time of blood sampling, and any post-dosing complaints.

3.3. Pyrazinamide dose and blood sampling:

Study participants were kept *nil per os* from 12pm prior to the sampling day. On the morning of the sampling, baseline blood samples were taken for liver and renal function tests, haematological and virological tests. Patients were then given their usual dose of anti-TB medications, including PZA, and the time was noted. They were then allowed to eat and drink as normal. All patients received single dosage forms (i.e. not fixed dose combinations) at a dose of 25mg/kg.

Blood samples for anti-TB drugs plasma concentrations were collected from an intravenous catheter placed in a forearm vein of each patient and placed in heparinized tubes before being centrifuged at 5,250 rpm for 5 minutes. Using a micropipette, the plasma was separated and stored at -80°C until the date of analysis.

3.4. Laboratory tests:

Haematological tests (white blood cell count, red blood cell count, haemoglobin, and platelets), liver function (ALT, AST, GGT, and bilirubin) and renal function (urea and electrolytes, creatinine) tests were performed by PathCare Laboratories (Worcester). Immunological and

virological tests (HIV test, CD4 count, viral load) were conducted by the National Health Laboratory Services (NHLS) at Tygerberg Hospital.

3.5 Determination of pyrazinamide plasma concentrations:

PZA plasma concentrations were analysed using liquid chromatography-mass spectrometry at the Central Analytical Facility at the University of Stellenbosch in the Western Cape.

The LC-MS analysis was performed using a Waters Accuity UPLC (ultra performance liquid chromatography) system connected to a Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA). The column utilized was a Waters BEH Phenyl (1.7µm, 2.1mm x 100mm) at ambient temperature. The mobile phase used was 0.1 % formic acid in water (v/v) as solvent A, solvent B consisted of 0.1% formic acid in acetonitrile. A flow rate of 0.3 ml/min was applied and an injection volume of 5 µl. The gradient started at 98% solvent A for the first 0.1 minutes followed by a linear gradient over 4 minutes to 60 % solvent B. The column was washed for 1 minute at 100% solvent B and re-equilibrated for another 6 minutes at the starting conditions.

The MS conditions were as follows: electrospray ionization in the positive mode was applied, the ion source and desolvation temperature were held at 100°C and 500 °C, respectively. The capillary voltage was 2.8 kV. The desolvation gas at 1000 L/h and the cone gas was 50 L/h. The instrument was operated at multiple reaction monitoring (MRM) mode. The MRM settings for PZA was 124>107 at collision energy 10 eV and cone voltage of 20 V. Propanolol was used as internal standard and was monitored at an MRM of 260.3>183 at a collision energy of 20eV and

cone voltage of 18 V. Waters MassLynx™ software was used for the data collection and processing.

All chemicals used were obtained from Sigma-Aldrich (Cape Town, Western Cape).

3.5.1 Preparation of standards

The stock solution of 1 mg/ml was serially diluted with acetonitrile to obtain working solutions with the concentrations 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 µg/ml. These standards all contained 10 ppm propanolol as an internal standard.

3.5.2 Patient samples preparation

To prepare the patients' plasma samples for the LC-MS assay, trichloroacetic acid (30 µl) was added to 50 µl plasma followed by 170 µl internal standard solution (10 ppm propanolol in water). The mixture was vortexed for 1 minute, followed by centrifugation at 6000 g for 5 minutes. [Jiang et al, 2011]. The supernatant was injected onto the LC-MS. Blank plasma was spiked with 1 and 5 ppm of PZA in triplicate to determine the recoveries and repeatability. The relative standard deviation was better than 7%.

3.5.3 Pyrazinamide tablets

PZA tablets (500mg, Batch No 1G029A, Sanofi Aventis, SA) were supplied by BKH and used as working standards.

3.6 Pharmacokinetic Analysis:

Pharmacokinetic data for both groups was obtained by plotting the observed concentrations vs. time using GraphPad Prism 6 software (GraphPad Software, Inc, California).PZApharmacokinetic parameters were calculated based on the non-compartmental analysis.

- The maximum plasma concentration (C_{max}) was obtained directly from the plasma concentration-time profile

- The time to reach the maximum plasma concentration (T_{max}) was also obtained directly from the plasma concentration-time profile

- The elimination rate constant (K_e) was calculated as –2.303 multiplied by the slope of the terminal log-linear phase of the plasma concentration-time profile

- The half-life ($T_{1/2}$) was calculated as $0.639/K_e$

- The area under the plasma concentration vs. time curve from 0 to 24 hours (AUC_{0-24}) was calculated by the trapezoidal method using GraphPad Prism software

- The volume of distribution (V_d) was calculated as Dose/(AUC x K_e)

- The total body clearance (Cl_{tot}) was calculated as Dose/ AUC

- The area under the plasma concentration vs. time curve from 0 to infinity ($AUC_{0-\infty}$) was calculated using the equation: $AUC_{0-\infty} + Cp_{last} / K_e$

- The volume of distribution (V_d) was calculated as Dose/($AUC_{0-\infty}$ x K_e)

- The total body clearance (Cl_{tot}) was calculated as Dose/ $AUC_{0-\infty}$

- The mean residence time (MRT) was calculated as: MRT= $AUMC_\infty/AUC_\infty$

Where $AUMC_\infty$ is the area under the momentum curve from zero to infinity calculated as

$AUMC_\infty = AUMC + C_{last}/K_e + (t_{last} \times C_{last})/ K_e$

AUMC is the area under the momentum curve and calculated as

AUMC = (tici+ti-1 x ci-1)/2 x (ti-ti-1)

3.7 Statistical analysis:

The data that was obtained during the study was captured and stored using Microsoft Excel for Windows. Results were reported as medians and ranges, due to the fact that the sample size was relatively small and the results were fairly skewed. Comparisons were done using the nonparametric Wilcoxon Rank Sum test, using a level of significance of 0.01.

Comparisons were done using the nonparametric Wilcoxon Rank Sum test, using a level of significance of 0.01.

3.8 Ethical considerations:

This study was registered and approved by the University of the Western Cape's ethics committee under the ethics clearance registration number 07/6/12.

Permission was granted from the provincial department of health, as well as from the medical superintendent of Brewelskloof Hospital. The study was conducted according to the declaration of Helsinki and ICH guidelines. All patient information remains confidential.

3.9 Limitations of the study methods

The study was limited by the amount of staff and help that were able to assist in blood collection. It would have been preferable to be able to take more samples, and hence have more data points, especially in the first few hours after dosing, in order to obtain a more accurate pharmacokinetic picture. Although we were able to calculate all required pharmacokinetic parameters, differences and extreme values may have been over or under-estimated due to longer time intervals between data points. In addition, there was some difficulty in drawing blood from a few patients, which compromised the times slightly. The demographics of the patient population was limited by the number of patients available at that specific time – although attempts were made to recruit similar numbers of male and female, HIV positive and negative etc, this was not always possible, and subsequently resulted in an unbalanced sample size. The size of the study was limited by financial constraints, the evaluation of the true effect of HIV on the pharmacokinetics of PZA would require a higher number of patients in order to provide a statistically significant comparison.

CHAPTER FOUR:

RESULTS

4.1 Demographic characteristics of the study population

A total of 32 patients were enrolled in this study. Out of 32 patients, 9 were HIV positive (28%), and 23 (72%) were HIV negative. The median dosage of PZA and the median patient weight were comparable in both populations.

Table 6: PZA dosage and patient weight:

Patient population:	Median Dose:	Median weight:	Median Dosage/kg:
Positive	1500mg	57.24kg	26.2mg/kg
Negative	1500mg	51kg	24.4mg/kg

Table 7: Demographics of study population:

Study participants:	Number:	Percentage of total:
Female	14	43.75%
Male	18	56.25%

Of the HIV - positive patients, 4 had a CD4 count greater than 200, with a median CD4 count of 354.5.

5 of the HIV positive patients had a CD4 less than 200, with a median CD4 of 91.

Table 8: HIV status of study population:

HIV status:	Number:	Percentage of total:
Negative	23	71.9%
Positive, CD4 count > 200	4	12.5%
Positive, CD4 count < 200	5	15.6%

4.2 Pyrazinamide LC-MS analysis method

The LC-MS method for the determination of PZA in human plasma was developed and validated. The validation of PZA LC-MS analysis was conducted by determining linearity, recovery, precision and accuracy, low limit of detection, low limit of quantification and specificity.

4.2.1 Calibration curve and linearity

The calibration curve was constructed by plotting the responses (peak areas) of PZA against the corresponding concentrations. The correlation coefficient (r^2) of determination of PZA during the validation was greater than 0.99.

4.2.2 Precision and accuracy

PZA recovery was determined by comparing peak areas from the plasma samples that spiked with PZA after extraction (i.e. calibration standards) with the corresponding PZA standard solutions. The percentage recovery of PZA was 84.1 ±6.7% at 1ppm spike and 74.7 ±4% at 10 µg/ml.

4.2.3 Lower limit of detection and quantification

The lower limit of detection (LOD) of PZA plasma concentration that could be determined (taking into consideration the concentration which produces a signal to ratio of 3 that gives a measurable response from the MS detector) was 0.06 µg /ml. PZA lower limit of quantification (LOQ) in the plasma was found to be 0.10 µg /ml.

4.2.4 Specificity

There were no interfering peaks from the plasma components with the PZA peak, which was detected at a retention time of 9.68 minutes. Chromatograms showing the separation of PZA plasma extract are shown in Figure 2.

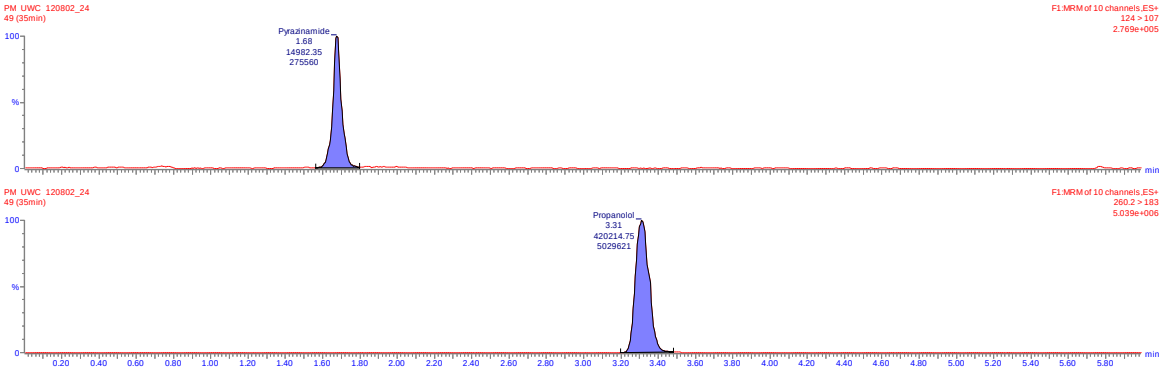

Figure 2: Chromatogram of pyrazinamide plasma extract

4.3 Pharmacokinetic data

The results obtained are summarized in the graphs and tables below.

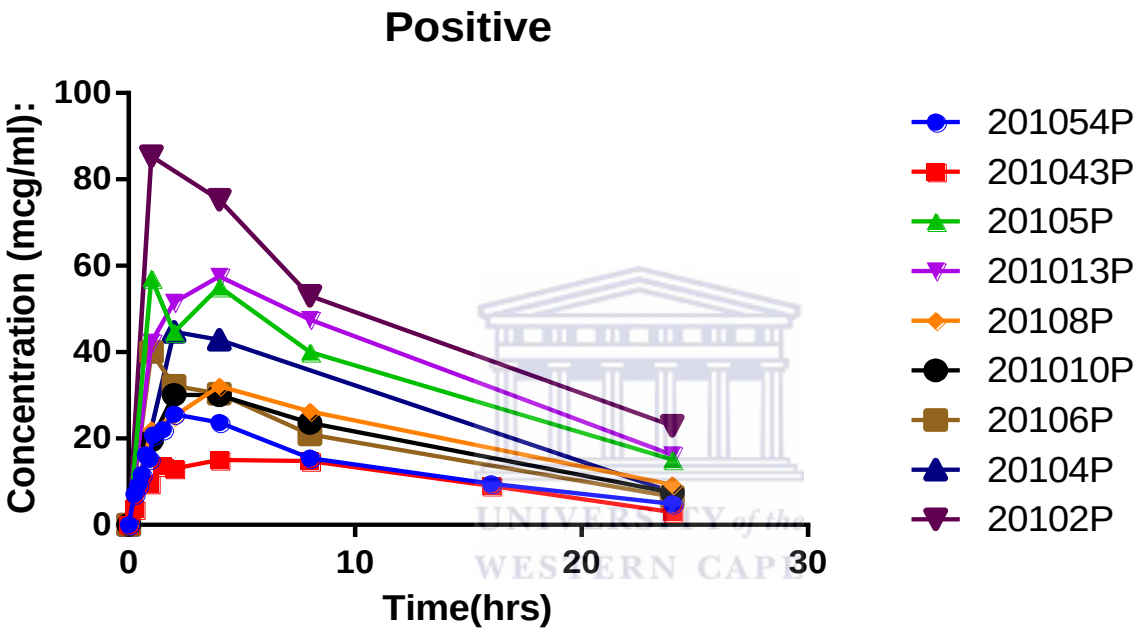

Figure 3: Concentration vs. Time profiles for HIV positive patients

(Where the numbers 201054P, 201043P etc denote the study numbers allocated to HIV positive study participants.)

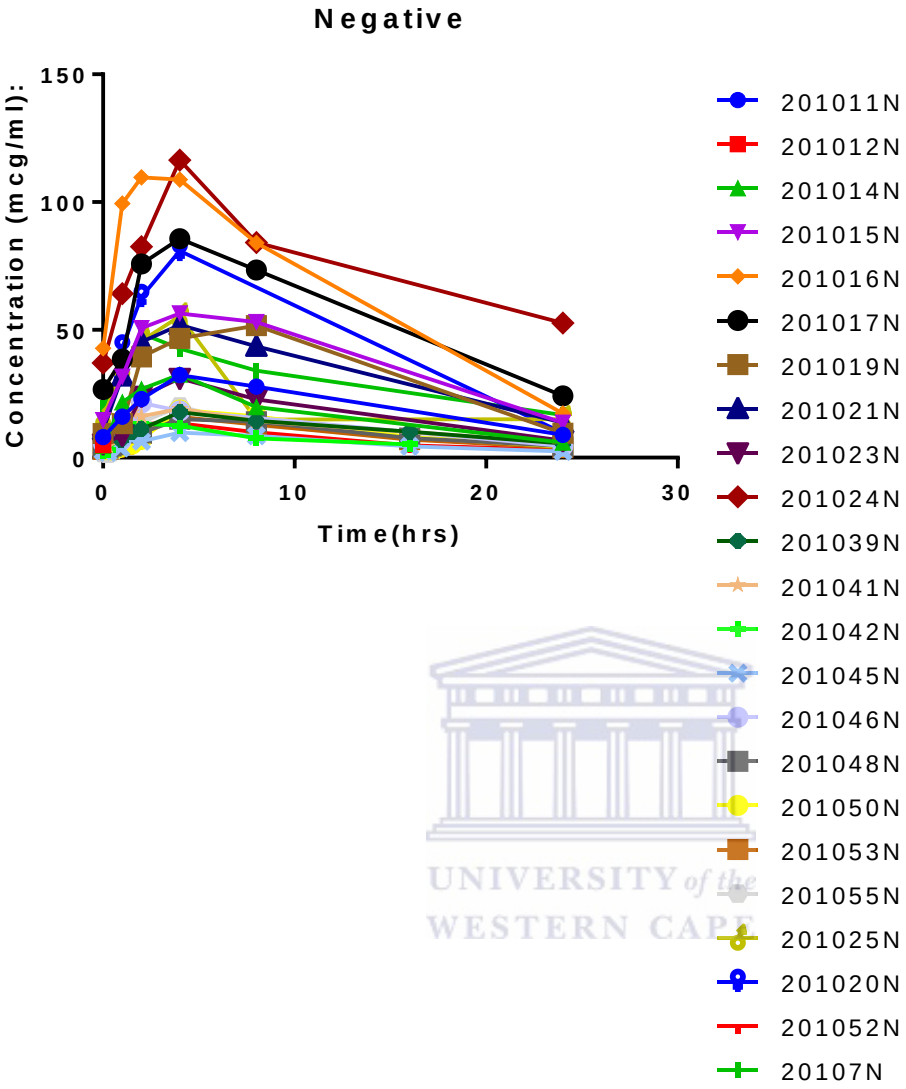

Figure 4: Concentration vs. Time profiles for HIV negative patients:

(Where the numbers 201011N, 201012N etc denote the study numbers allocated to HIV negative study participants.)

Table 9: Median (range) Pharmacokinetic Parameters:

	HIV Positive	**HIV Negative**
C_{max} (mcg/ml)	40.04 (70.35)	32.34 (106.62)
T_{max} (hours)	2 (3)	4 (6)
K_e	0.0664 (0.02658)	0.07183 (0.05998)
$T_{1/2}$	10.44 (3.95)	9.65 (8.44)
AUC_{0-24}	493.4 (912.1)	420.3 (1434.8)
$AUC_{(24-\infty)}$	112.5 (362.96)	90.87 (1401.07)
$AUC_{(0-\infty)}$	639.9 (1261.66)	507.83 (3060.57)
V_d	24.87 (52.86)	29.74 (79.71)
Cl	2.06 (4.61)	1.97 (6.45)
MRT	8.60 (3.44)	8.63 (4.92)
K_a	0.84 (0.66)	0.5 (1.02)

Table 9 above shows the median pharmacokinetic parameters for both HIV-positive and HIV-negative patients. The median $AUC_{(0-\infty)}$,, C_{max}, half life, K_a and clearance values were higher in the HIV positive population than in the negative patients.

HIV negative patients had a higher T_{max} and K_e than positive patients, and showed an increased apparent volume of distribution. Mean residence times were similar in both populations.

4.4 Statistical analysis

The pharmacokinetic parameters of the two groups were compared using the nonparametric Wilcoxon Rank Sum test and the level of significance was set at 0.01.

The findings of this analysis are shown in Tables 10, 11 and 12 below.

Table 10: The MEANS Procedure – All Patients (HIV negative and HIV positive):

Variable	N	Mean	Median	SD	Lower Quartile	Upper Quartile	Minimum	Maximum
$AUC_{(0-\infty)}$	32	775.8525	563.9200	631.1487	330.9300	1067.6200	175.1900	3235.7600
C_{max}	32	42.0916	32.4300	28.2230	18.9400	55.8800	9.800	116.4200
T_{max}	32	3.3438	4.0000	1.4053	2.0000	4.0000	1.0000	8.0000
Ke	32	0.0711	0.0712	0.0151	0.0631	0.0773	0.0367	0.1063
$T_{1/2}$	32	10.2341	9.7400	2.4831	8.9650	10.9950	6.5200	18.8800
V_d	32	39.5941	27.7150	25.2899	19.6850	62.4250	8.4200	88.1200
Cl	32	2.7813	2.0150	1.8078	1.4050	4.6550	0.3100	6.7600
MRT	32	8.4813	8.6150	1.1685	7.8500	9.2400	5.4500	10.3700
K_a	32	0.6275	0.5700	0.2710	0.4100	0.8400	0.3000	1.32

Table 11: The MEANS Procedure – HIV Negative Patients:

Variable	N	Mean	Median	SD	Lower Quartile	Upper Quartile	Minimum	Maximum
$AUC_{(0-\infty)}$	23	778.9996	507.8300	706.8521	303.9100	1056.2200	175.190	3235.7600
C_{max}	23	41.7035	32.3400	30.9646	17.8000	55.3100	9.8000	116.4200
T_{max}	23	3.7391	**4.0000**	1.2511	4.0000	4.0000	2.0000	8.0000
K_e	23	0.0717	0.0718	0.0168	0.0630	0.0780	0.0367	0.1063
$T_{1/2}$	23	10.2570	9.6500	2.8079	8.8900	11.0100	6.5200	18.8800
V_d	23	42.1039	29.7400	27.8790	18.2300	68.7000	8.4200	88.1200
Cl	23	2.9355	1.9700	1.9438	1.4200	4.8600	0.3100	6.7600
MRT	23	8.5217	8.6300	1.2557	7.5000	9.5700	5.4500	10.3700
K_a	23	0.5609	0.5000	0.2547	0.3400	0.6800	0.3000	1.3200

Table 12: The MEANS Procedure – HIV Positive Patients:

Variable	N	Mean	Median	SD	Lower Quartile	Upper Quartile	Minimum	Maximum
$AUC_{(0-\infty)}$	9	612.1111	639.9	299.9956	443.5000	823.6000	249.9000	1162.0000
C_{max}	9	4.0833	40.0400	21.1738	30.1600	57.1600	15.0400	85.3900
T_{max}	9	2.3333	**2.0000**	1.3229	1.0000	4.0000	1.0000	4.0000
K_e	9	0.0694	0.0664	0.0101	0.0631	0.0764	0.0563	0.0828
$T_{1/2}$	9	10.1756	10.4400	1.4851	9.0700	10.9800	8.3700	12.3200
V_d	9	33.1800	24.8700	16.6303	24.7100	40.0600	11.2900	64.1500
Cl	9	2.3878	2.0600	1.4252	1.3900	2.6600	0.6500	5.2600
MRT	9	8.3778	8.6000	0.9683	8.4200	8.8200	6.1100	9.5500
K_a	9	0.7978	0.8400	0.2469	0.6000	1.0000	0.4400	1.1000

Table 13 : P-values for Wilcoxon tests

Parameter:	P-value:
C_{max}	0.5342
T_{max}	**0.0117**
K_e	0.7397
$T_{1/2}$	0.7397
Vd	0.6480
Cl	0.6780
$AUC_{(0-\infty)}$	0.4563
MRT	0.6780
K_a	**0.0241**

CHAPTER FIVE:

DISCUSSION

We were able to successfully fulfill all of our specific objectives as outlined in the beginning of our study. On the basis that previous authors had found evidence of altered pharmacokinetics of anti-TB drugs in certain patients, and that the pharmacokinetics of PZA has been incompletely characterised [Perlman et al, 2004], our study aimed to investigate and describe the pharmacokinetics of PZA in patients with MDR-TB and in patients with MDR-TB/HIV-infection. There are very few studies in the available literature that utilise LC-MS to assay plasma levels of anti-TB drugs, and our study also aimed to investigate whether or not this method could be used to analyse PZA levels in the plasma.

As highlighted earlier, there is evidence to suggest that some patients (both with and without HIV) might be subject to subtherapuetic levels of anti-TB drugs, possibly as a result of malabsoprtion or drug interaction, although this phenomenon seems to be more frequent in HIV positive patients than negative. Peloquin et al (1993), shared their experience of the analysis of over 1000 assays of anti-TB drug plasma levels over a six-month period at their facility in Colorado. Using HPLC or GC, they observed that most (70%) of the plasma concentrations in these samples were below normal reference values for 10 commonly used anti-TB drugs, including PZA (Peloquin, 1992). Peloquin and his team of researchers at the National Jewish Medical and Research Centre in Colorado have done much work in this field. They have conducted extensive analyses of serum drug levels of the TB drugs in both patients with and without TB, and their work has shown that the degree of TB-drug malabsorption differs in

different population groups. Although the exact causes of drug malabsorption are not well defined, they point out that it is reasonable to contemplate this phenomenon in patients co-infected with HIV and TB. They propose that where malabsorption is suspected, serum drug levels taken at 2 hours post-dosing and 6 hours post-dosing may help to confirm this and also help distinguish between malabsorption and delayed absorption. They believe that low serum concentrations of the anti-TB drugs may be related to poor clinical outcome, but agree that there is much more research needed in this field and encourage researchers in other parts of the world to pursue and investigate this [Peloquin et al, 1999]. The cause of malabsorption in HIV positive patients may be linked to HIV-associated enteropathy [Wilson et al, 2008]. Pozniak et al [1999] also agreed that malabsorption should be suspected in any patients (HIV positive or negative) who fail to respond to therapy despite good adherence to treatment.

Despite these findings, there are several conflicting reports that find the opposite to be true. Taylor and Smith [1998] investigated the pharmacokinetics of rifampicin, isoniazid and PZA in a small cohort of South African TB patients. They used HPLC to determine the plasma concentrations and concluded that HIV was not associated with lower concentrations of the 3 drugs. Wilkins et al [2006] used HPLC with UV detection to study the pharmacokinetics of PZA in South African patients with DS-TB. They described a "one compartment model with first-order absorption…and first-order elimination". The study revealed that although 2 distinct patient groups emerged from the data ("fast" absorbers and "slow" absorbers), these all fell within normal estimates, and they concluded that the pharmacokinetics of PZA did not vary significantly between different patient populations.

Several other researchers also failed to prove an association between AIDS and TB drug malabsorption. Choudhri et al [1997] concluded that the presence of AIDS or diarrhoea did not influence the pharmacokinetics of PZA, rifampicin and isoniazid in Kenyan patients. Conte et al [1999] reported that although plasma and intrapulmonary concentrations of PZA were lower in non-TB-infected AIDS patients than in healthy volunteers, this difference did not reach statistical significance. They found the concentrations of PZA in epithelial lining fluid to be far greater than the plasma concentrations, and hypothesise that these high concentrations in the lungs might be a reason for the success of the drug in the treatment of PTB. Perlman et al [2004], studied the pharmacokinetics of PZA in 100 patients co-infected with HIV and TB. They utilized HPLC and having defined a low cut-off concentration of 25mg/L for PZA, they found that low serum levels were uncommon. All of these studies were conducted in patients with DS-TB; we failed to find any studies in South Africa that examined the differences in patients with DR-TB.

We were able to effectively establish and calculate the required pharmacokinetic parameters in both study groups by using the plasma concentration values found in our analysis. In our study, the median $AUC_{(0-\infty)}$, C_{max}, $T_{1/2}$ and clearance values were higher in the HIV positive population than in the negative patients, although none of these differences were statistically significant. HIV negative patients had a higher T_{max} than positive patients, and showed an increased apparent volume of distribution. Mean residence times were similar in both populations. Our findings supported the views of Taylor and Smith [1998], and Wilkins et al [2006] above in that we found no significant differences between the pharmacokinetic parameters of PZA in HIV positive and negative patients with MDR-TB. The only exception to this was with regards to T_{max} ($p=0.0117$), indicating that HIV negative patients with MDR-TB took significantly longer to reach maximum

concentrations of PZA in the plasma. However, the importance of this finding may be over-estimated in this study due to the logistical limitations affecting the number of sampling times. PZA is rapidly absorbed, with T_{max} often occurring around 1 hour [Peloquin et al, 1998]. More samples in the first 2 hours would have been possible with more resources and this could have painted a more accurate picture of the pharmacokinetic profiles, and therefore of the parameters that were calculated.

Although there was a difference in K_a between the 2 populations, with HIV positive patients having a higher level than HIV negative, this difference did not quite reach statistical significance at the 0.01 level (p=0.0241). For PZA, Peloquin suggests a target C_{max} of 20 to 40 mcg/ml after a 25 mg/kg daily dose [Peloquin, 2002]. In our study, HIV positive patients had a median dose of 26.2mg/kg/day and a median C_{max} of 40.04mcg/ml. HIV negative participants had a median daily dose of 24.4mg/kg with a median C_{max} of 32.34mcg/ml. Hence, both groups of patients in our study achieved C_{max} values within the desired range. In a study in Botswana by Tappero et al [2005], the influence of HIV on the serum concentrations of the antimycobacterial drugs was investigated. It was found that although low serum levels of rifampicin, isoniazid and ethambutol were common, PZA was well absorbed. They defined a low C_{max} as less than 35mcg/ml and a very low C_{max} as 20mcg/ml. They found that HIV-positive patients had longer half-lives for PZA than HIV-negative patients, and this is in agreement with our findings. Although PZA levels were lower in the HIV-positive group in their study, the levels still fell within normal ranges. Since treatment for drug resistant TB is limited by the decreased effectiveness and toxicity of the second-line agents [Kahana, 1996], it makes sense that we investigate the available drugs fully, and ensure that they reaching therapeutic levels in the

serum, while limiting their toxic side effects. TDM involves the use of serum concentrations to adjust a patient's dose to obtain a desired serum concentration (ideally one which is therapeutic, while minimizing side effects). While TDM is a useful tool, its use is limited by the fact that it is logistically expensive. Yew [2001], points out the usefulness of TDM in the management of TB, particularly in patients with poor clinical outcomes and patients in whom subtherapeutic drug levels may occur. He also highlights the usefulness of TDM in TB therapy when there is the possibility of drug-drug interactions and drug-disease interactions. This may be especially pertinent with the use of rifampicin, a potent cytochrome P450 inducer or in patients with hepatic or renal impairment, or those with other disease states that might influence drug kinetics, such as HIV. Mehta et al [2001] identified subtherapeutic levels of rifampicin in patients who were slow to respond to standard anti-TB treatment. After increasing their dose to reach a therapeutic plasma level, all patients showed a clinical and mycobacteriological response. The authors suggest that such interventions should be considered in patients with HIV, malnutrition, known GI or malabsorptive disease, and hepatic or renal disease.

Peloquin [2004] explains that although there are logistical difficulties in implementing TDM for TB patients, it may help to maximize therapy in patients with TB and MDR-TB and in particular in patients for whom the published dosing guidelines do not elicit the necessary serum concentrations or clinical response. He expresses concern over patients who exhibit malabsorption of the anti-TB drugs, and suggests in these cases, that TDM might be useful in helping to optimize therapy and minimize treatment failures [1998]. He also makes some pertinent observations about the doses of anti-TB drugs, saying that in some patients, doses above the "normal" ranges may be needed, and that well-timed serum drug level observations

may help the practitioner to establish the correct dose for an individual patient who may be failing treatment due to subtherapeutic serum concentrations [2001].

It is inadequately studied whether or not low levels of the anti-TB drugs impact on a patients' clinical outcome. Chideya et al [2009], looked at the pharmacokinetics of the first-line drugs and how they related to treatment outcomes, in 225 Botswanan patients, most of which were HIV-positive. Low levels were found for 84% of patients for rifampicin, 37% for isoniazid, 39% for ethambutol and 5% for PZA. Of the four drugs, only low PZA level was associated with poor treatment outcomes in both HIV positive and negative patients. Positive HIV status with CD4 count less than 200 was also related to poor treatment outcome, and they concluded that patients with a low PZA C_{max} were 3 times more likely to develop poor treatment outcomes than patients with a normal C_{max}. Patients were dosed at 35mg/kg/day and low C_{max} was defined as concentrations less than 35mcg/ml.

Another study of TB patients in Botswana produced some interesting findings. HIV positive patients with a CD4 count less than 200 were found to have a higher risk of poor treatment outcome than those that were HIV negative or those that were HIV positive but had a CD4 count greater than 200 [Chideya et al, 2009]. Patients with a low PZA C_{max} were 3 times more likely to have a poor treatment outcome than patients with a normal C_{max}, and PZA C_{max} was found to be significantly lower in patients with a CD4 < 200 than in patients with a CD4 > 200, regardless of the patient's HIV status. Patients with good treatment outcomes showed PZA C_{max} values in the higher ranges, while lower PZA C_{max} values were associated with poor treatment outcome.

This study sheds important new light on the pharmacokinetics of PZA in MDR-TB patients. PZA plays a crucial role in the treatment of DS-TB and DR-TB due to the fact that it is active against dormant tubercule bacilli and high levels of MDR-TB are already prevalent in some parts of South Africa [Cox et al, 2010). With the cure rate amongst patients with MDR-TB known to be as low as 32% [Kritski et al, 1997] and the emergence of XDR-TB which is notoriously deadly and hard to cure, with mortality rates around 40% and cure rates as low as 22% [O'Donnell:2013], it is crucial that we gather as much information as possible, in an attempt to optimize treatment and curb the spread of the disease.

Peloquin et al [1999], who have conducted extensive analyses on the anti-TB drug serum levels in different patient populations, agree that although the risk factors for, and extent of malabsorption is largely unknown, it is reasonable to suspect that it may occur in patients with HIV/AIDS, and may play a role in subtherapeutic serum drug levels. Whether or not these low serum levels translate to poor clinical outcomes is largely unknown, and he encourages fellow researchers to investigate this more fully.

Our study also aimed to investigate whether or not LC-MS could be used to analyse PZA levels in the plasma. A LC-MS method for the determination of PZA in plasma was developed and validated in our study, with the validation having been conducted by determining linearity, recovery, precision and accuracy, low limit of detection, low limit of quantification and specificity. The method that was developed was linear, sensitive, specific, precise and accurate, and was therefore suitable for the quantitative analysis of PZA in our study. It was difficult to compare our results with previous studies, as there are not many studies using LC-MS to analyse PZA levels in the plasma, with most of the examples in the literature being HPLC and less

frequently, GC. Um et al [2007] had also developed a LC-MS/MS method for the determination of the TB drugs in plasma, and used this to evaluate serum levels in patients with TB. They found low concentrations of the antiTB drugs to be common, with rifampicin having the highest prevalence with 23.5% and PZA the lowest with 4.5%. Song et al [2007] were also able to develop a method for analyzing PZA using LC-MS. Our study successfully used LC-MS, with our plasma level analysis results being similar to those found in other studies using methods such as HPLC [Taylor and Smith, 1998; Wilkins et al 2006].

CHAPTER SIX:

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, we were able to achieve the objectives set out for this study and our research was able to characterise the pharmacokinetics of PZA in both patients with MDR-TB and patients with MDR-TB and HIV. This was successfully achieved by developing and validating an LC-MS assay method for the determination of PZA levels in human plasma, and we therefore accept our first experimental hypothesis. We found no evidence of a significant difference between the two population groups with regards to any of the pharmacokinetic parameters studied, the exception being T_{max}, and therefore reject our second experimental hypothesis and accept the null hypothesis in this case.

Our research was limited by a relatively small overall sample size, and an unbalanced distribution between the 2 patient groups, as well as the inability to compare our results with those in healthy volunteers. The lack of previous similar studies utilizing LC-MS also restricted our ability to compare our results with similar populations. Notably, the scope of this study did not extend to explore the influence of PZA plasma concentrations on clinical outcome, which is the ultimate goal and important measurable result for both the individual patients, and the epidemiology of the disease. This study also did not investigate the influence of HIV infection on fAUC/MIC ratio, which is again an important measurable endpoint.

There is much more research needed in the area of pharmacokinetics of antimycobacterial drugs in MDR-TB patients, especially in South Africa. Even greater, is the need to explore these pharmacokinetics in the HIV positive population, as these patients make up a significant

proportion of our MDR-TB patient population in South Africa. Further investigation is required in order to establish if these patients are being exposed to subtherapeutic or toxic doses, and whether or not this has an impact on treatment outcome.

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