THE RELATIONSHIP BETWEEN THE BLOOD CONCENTRATION OF ISONIAZID, RIFAMPICIN, PYRAZINAMIDE AND THE TREATMENT OUTCOMES OF PULMONARY TUBERCULOSIS PATIENTS

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UNIVERSITI SAINS MALAYSIA

2017

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by

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Thesis submitted in fulfillment of the requirements
for the degree of
Doctor of Philosophy

April 2017

DEDICATION

I dedicate this thesis to my late father Abdallah Sulaiman Mahjoub, my mother Khadija Abdulsalam, my brothers Hasan Abdallah Mahjoub and Jamal Abdallah Mahjoub, my wife Mariam Mohamed, and my children Khadija Abdallah Mahjoub, Abdulrahman Abdallah Mahjoub, and Jenan Abdallah Mahjoub

ACKNOWLEDGEMENT

First of all, I am very grateful to almighty ALLAH the most gracious; most merciful for the wisdom and perseverance that He has given me throughout my whole life and especially during the period of my PhD study. I would like to express my appreciation to everyone who supported and helped me in conducting and completing this research. I would like to express my deepest gratitude and appreciation to my supervisor Prof. Dr. Syed Azhar Syed Sulaiman and my co-supervisors Assoc.Prof. Dr. Razak Lajis and Dr. Amer Hayat Khan for their guidance support, and valuable advises which were the spirit that encourage me for completing this research.

I would also like to thank my field supervisor Dr. Irfhan Ali ben Hyder Ali, Head of Respiratory Department in Hospital Pulau Pinang, and all the medical staff at Respiratory Ward and Clinic at Hospital Pulau Pinang for their guidance and support. Very special thanks to the staff nurses Anizasazila binti Mohamed Nazri and Noor Izura binti Mohamed Rodzi. I would also like to extend my thanks to all the patients who participate in this study.

I would like to express my gratitude to my field supervisor Mrs. Che Nin Man, the Senior Science Officer in the Toxicology Laboratory Unit at the National Poisoning Center, and to all the other staff in the Toxicology Laboratory Unit specially Mrs. Norjuliana Mohd Noor for her assistance and help.

I would like to express my deepest gratitude and thankfulness to the Faculty of Pharmacy, Misurata University and the Ministry of Higher Education and Scientific research, Libya for giving me the scholarship to pursue my education. I would also

like to thank the Universiti Sains Malaysia for opening the door to me and giving me such a valuable opportunity to continue my academic education.

I would like to thank all my family members especially my mother Khadija Abdulsalam, and my elder brothers Hasan Abdallah Mahjoub, and Jamal Abdallah Mahjoub for all the care and love that they have given me throughout my life and the right morals and ethics they have though me. I also would like to thank my my wife and my children Khadija, Abdulrahman, and Jenan for always being with me.

TABLE OF CONTENTS

CHAPTER 3: METHODOLOGY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

ADR	Adverse drug reaction
AFB	Acid-fast bacilli
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
ARR	Acquired rifamycin resistance
AUC	Area under the concentration-time curve
BC	Before Christ
C°	Celsius
C_{2h}	Concentration at 2hours post-dose
CBC	Complete blood count
CD4	Cluster of differentiation4
CFU	Colony forming units
CI	Confidence interval
C_{max}	Maximum concentration
CRC	Clinical research center
CS	Culture and susceptibility
CS	Calibration standards
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid

DOTS	Direct observed treatment short-course
EBA	Early bactericidal activity
EMB	Ethambutol
EPTB	Extra-pulmonary tuberculosis
FDC	Fixed-dose combination
h	Hour
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
INH	INH
IQR	Interquartile range
IS	Internal standard
IU	International unit
K_2 EDTA	Potassium Ethylenediaminetetraacetic acid
kg	Kilogram
L	Liter
LLOQ	Lower limit of quantification
LOD	Limit of detection
LTB	Latent tuberculosis
M	Molar
MDR	Multi-drug resistant
mg	Milligram
MIC	Minimum inhibitory concentration
min	Minute
mL	milliliter
mM	Millimolar

MREC	Medical Research and Ethics Committee
NAT2	N-acetyl transferase 2
nm	Nanometer
QC	Quality control
OR	Odds ratio
PAS	Para-aminosalicylic acid
POA	Pyrazinoic acid
PTB	Pulmonary Tuberculosis
PZA	Pyrazinamide
PZase	Pyrazinamidase
RE	Relative error
rho	Spearman's correlation coefficient
RIF	Rifampicin
RNA	Ribonucleic acid
rpm	Revolutions per minute
RR	Relative risk
RSD	Relative standard deviation
SCC	Short-course chemotherapy
SD	Standard deviation
SM	Streptomycin
S/N	Signal to noise ratio
TB	Tuberculosis
TB1	Thiacetazone
TCA	Trichloroacetic acid
TDM	Therapeutic drug monitoring

T_{max}	Time to reach maximum concentration
TNF α	Tumor necrosis factor alpha
UK	United Kingdom
ULN	Upper limit of normal
UV	Ultra-violet
WHO	World Health Organization
µL	Microliter

LIST OF APPENDICES

HUBUNGAN DI ANTARA KEPEKATAN ISONIAZID, RIFAMPICIN, PYRAZINAMIDE DALAM DARAH, DAN HASIL RAWATAN DALAM KALANGAN PESAKIT BATUK KERING (TUBERKULOSIS PULMONARI)

ABSTRAK

Sebuah kajian kohort prospektif dijalankan di Hospital Pulau Pinang bagi mengenal pasti penentu farmakokinetik INH (INH), rifampicin (RIF), and pyrazinamide (PZA), dan menilai kesan parameter farmakokinetik tersebut keatas dapat rawatan dalam kalangan pesakit tuberculosis pulmonari. Kepekatan INH, RIF, dan PZA plasma disukat menggunakan kaedah HPLC baharu yang dibangunkan dan pengesahannya adalah sebahagian daripada kajian ini. Median maksimum (C_{max}) kepekatan INH, RIF, dan PZA darah adalah masing-masing 4.75, 6.85, dan 42.00 mg/L. Median keluasan keluk masa-kepekatan dari sifar hingga 24 jam (AUC_{0-24h}) bagi INH dan RIF masing-masing adalah 20.10, dan 33.22 mg × h/L. Log INH C_{max}, dan log INH AUC_{0-24h} adalah ketara lebih tinggi sebanyak 33% (P = 0.000), dan 25% (P = 0.000) bagi pesakit berbangsa India berbanding bangsa lain. Log INH C_{max} dan log INH AUC_{0-24h} mengalami pengurangan sebanyak 20% (P = 0.042), dan sebanyak 23% (P = 0.000) dalam kalangan pesakit yang ketagihan alkohol. Log RIF C_{max} dan AUC_{0-24h} bagi RIF adalah lebih tinggi sebanyak 14% (P = 0.018), 23% (P = 0.026) bagi pesakit diabetes. RIF AUC_{0-24h} ketara lebih rendah bagi pesakit ketagih alkohol sebanyak 27.6%, P = 0.042. Log PZA C_{max} menujukkan peningkatan ketara sebanyak 5%, (P = 0.004), bagi pesakit berbangsa India. Di penghujung fasa intensif, 84.7% pesakit yang pada awalnya positif ujian calitan memberikan keputusan calitan negatif. Pengubahan keputusan calitan kahak adalah signifikan dan memperoleh kesan positif daripada ukuran dasar berat badan pesakit (OR terselaras = 1.24; 95%

CI = 1.05 - 1.48, P = 0.011), kepekatan dasar protein total (OR terselaras = 1.20; 95% CI = 1.02 - 1.41, P = 0.026), dan memperoleh kesan negatif akibat merokok (OR terselaras = 39.8%; 95% CI = 1.55 - 1023.2, P = 0.026). Kesan advers ubat (ADR) dikesan pada 29.2% pesakit. Insidens ADR ketara lebih tinggi pada pesakit bukan perokok (OR terselaras = 4.40; 95% CI = 1.39 - 13.94, P = 0.012), dan pada pesakit yang diberi INH C_{2h} melebihi 3.4 mg/L (OR terselaras = 3.75; 95% CI = 1.18 - 11.96, P = 0.025). Sebagai rumusan tidak terdapat satu hubungkait yang signifikan antara parameter farmakokinetik INH, RIF, dan PZA serta konversi calitan atau dapatan rawatan terakhir untuk tuberkulosis pulmonari. Walaubagaimanapun, insiden ADR untuk antituberkulosis adalah tinggi dalam kalangan pesakit yang mengambil INH C_{2h}. Kajian yang lebih mendalam adalah diperlukan sebelum pemonitoran terapeutik drug (TDM) bagi rawatan tuberkulosis pulmonari dijalankan.

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ABSTRACT

A prospective cohort study was conducted in Hospital Pulau Pinang to find out the determinants of the pharmacokinetics of isoniazid (INH), rifampicin (RIF), and pyrazinamide (PZA), and to assess the effect of these pharmacokinetic parameters on the treatment outcomes of pulmonary tuberculosis patients. Plasma concentration of INH, RIF, and PZA was measured by new HPLC methods developed and validated as part of this study. The median of maximum plasma concentration (C_{max}) of INH, RIF, and PZA was 4.75, 6.85, and 42.00 mg/L respectively. The median of the area under the time -concentration curve from zero to 24 hours (AUC_{0-24h}) of INH and RIF was 20.1 and 33.2mg × h/ L respectively. The log INH C_{max}, and log INH AUC_{0-24h} were respectively significantly higher by 33%, (P = 0.000), and 25% (P = 0.000) in Indian patients. Log INH C_{max} and log INH AUC_{0-24h} were respectively reduced by 20% (P = 0.042), and by 23% (P = 0.000) in alcoholic patients. Respectively, the log RIF C_{max} and the AUC_{0-24h} of RIF were higher by 14% (P = 0.018), 23% (P = 0.026) in diabetic patients. RIF AUC_{0-24h} was significantly lower in alcoholic patients by 27.6% (P = 0.042). Log PZA C_{max} was significantly higher by 5% (P = 0.004), in Indian patients. At the end of intensive phase 84.7% of the initially smear positive patients were converted to smear negative status. The conversion of positive sputum smear was significant and positively affected by the baseline patients' body weight (adjusted OR = 1.24; 95% CI = 1.05 – 1.48, P = 0.011), the baseline total protein concentration (adjusted OR =

1.20; 95% CI = 1.02 – 1.41, P = 0.026), and negatively affected by tobacco smoking (adjusted OR = 39.8%; 95% CI = 1.55 – 1023.2, P = 0.026). The final treatment outcomes were not significantly affected by any variable. Adverse drug reactions (ADRs) related to antituberculosis agents occurred in 29.2% of the patients. Incidence of ADRs was significantly higher in non-smoker patients (adjusted OR = 4.40; 95% CI = 1.39 – 13.94, P = 0.012), and in patients who had INH C_{2h} above 3.4 mg/L (adjusted OR = 3.75; 95% CI = 1.18 – 11.96, P = 0.025). In conclusion, there was no significant relationship between the pharmacokinetic parameters of INH, RIF, and PZA and the sputum smear conversion or the final treatment outcomes of pulmonary tuberculosis. Nevertheless, the incidence of anti-TB ADRs was significantly higher in patients with higher INH C_{2h}. Further studies are required before the therapeutic drug monitoring (TDM) can be utilized in the treatment of pulmonary tuberculosis.

CHAPTER 1

INTRODUCTION

1.1 Burden of tuberculosis

Tuberculosis (TB) is a potentially fatal infectious disease caused by *Mycobacterium tuberculosis*. Although the disease mostly affects the lung causing pulmonary tuberculosis (PTB), any other organs in the body may also be infected which leads to extra-pulmonary tuberculosis (EPTB) (WHO, 2015). Tuberculosis is an ancient disease; the earliest evidence of its existence is dated back to 8000 BC (Herzog, 1998). Nowadays, TB is still one of the most important infectious diseases causing death in the world. In 2014 alone, the World Health Organization estimated that the incidence of TB was 9.6 million cases which is equivalent to 133 cases per 100,000 individuals worldwide. More than half (58%) and about 28% of the estimated cases are residents in Asia and Africa, respectively (WHO, 2015). About one-eighth (12%) of the estimated cases of TB are also infected with human immunodeficiency virus (HIV) (WHO, 2015). In the same year, WHO estimated the disease prevalence as 13 million cases worldwide which was equivalent to 174 cases per 100 000 population. WHO also estimated that by the end of 2015, the prevalence rate of TB will decrease by 42% compared to 1990, which means that the target of halving the prevalence of TB is not likely to be met worldwide (WHO, 2015).

In 2014, as many as 1.1 million and 390,000 tuberculosis deaths occurred globally among HIV-negative and HIV- positive TB patients respectively. There are 16 deaths per 100,000 individuals of HIV-negative TB patients. However, when HIV patients are included, this figure rises to 21 deaths per 100,000 individuals. Between 1990 and 2013, there was a decline by 47% in the global mortality rate among HIV-negative patients. This decline in TB mortality is due to the implementation of direct observation treatment short course (DOTS) strategy which was launched by the WHO in 1995 and considered as the most important public health breakthrough of the decade in term of live saved. Implementation of DOTS strategy ensures the proper delivery of antituberculosis drugs to the patients, prevents defaulting of the patients and drug's misuse. Consequently DOTS enhances the cure of the patients and prevents the development of drug resistance (WHO, 2015).

According to the WHO report in 2014, the incidence and the prevalence of tuberculosis in Malaysia was about 103; (95%CI, 83 – 124), and 135 (95% CI, 63–232) patients per 100,000 population respectively. Mortality rate was estimated by the WHO as 8 (95% CI, 4.5 – 12) per 100,000 population. Overall treatment success rate including all case of tuberculosis was about 76% in 2013, which is relatively far from the global target of 85% set by the WHO (WHO, 2014). Furthermore, the treatment success rate in Malaysia for HIV-positive patients and previously treated patients was only 51% and 30% respectively (WHO, 2014). Although, the practice of DOTS strategy in Malaysia is high, ranging from 93% to 100% (Ministry of health, Malaysia, 2012), further efforts are required to achieve the global target of success rate.

1.2 Causes, types, and pathogenesis of tuberculosis

The causative organism of tuberculosis remained unknown until its discovery in 1882, by a German scientist. By using a special technique of staining, Mr. Robert Koch managed to see slender rods in the tuberculous tissue, which he called tubercle bacilli (Herzog, 1998). This bacillus is now known as *Mycobacterium tuberculosis,* the fundamental causative organism of tuberculosis disease. *M. tuberculosis* enters the host body by inhalation of infected air. When it reaches the lungs; it triggers the immune system as evident by tuberculin test. The immune system, especially the macrophage, with an assistant from CD4 lymphocytes, interferon gamma, and tumor necrosis factor alpha (TNFα) is able in 80% of the cases to eradicate all the bacteria that have entered the body (Peloquin, 2005). In the other cases, some of the bacilli are not killed but stay surrounded by a defensive barrier built by the immune system. In this case, these bacilli are not active, and the person is neither ill nor infectious. The *M. tuberculosis* might remain years in this dormant status, which is known as latent tuberculosis (LTB) (Peloquin, 2005; Barker, 2008; Knechel, 2009). In fact, about one-third of the world's population has LTB, but, fortunately, only 10% of them might develop an active TB disease in their lifetime (Barker, 2008).

Should the immune system fail in killing or capturing the invasive mycobacteria, the bacilli may spread within the lung, leading to pulmonary tuberculosis. The bacilli may invade the lymph glands within the chest leading to intra-thoracic respiratory tuberculosis or further spread through the blood stream and

affect any other tissue leading to EPTB (Peloquin, 2005). The active disease may also arise from the activation of the LTB many years after the original exposure (Peloquin, 2005; Barker, 2008; Knechel, 2009). Although *M. tuberculosis* can affect any tissue in the body, PTB is the most common and most infectious form of TB (Barker, 2008).

1.3 Risk factors for tuberculosis

Anyone who comes in close contact with pulmonary or laryngeal TB patients might get the infection. The disease is transmitted by inhaling the droplet nuclei that are produced when those patients cough, sneeze, talk or sing. As a result, people living in TB endemic area, people who have close contact with TB patients are more prone to get the disease (Peloquin, 2005). However, the risk of getting the infection and the active disease varies from one person to another according to some other factors, which include:

1. The bacillary load of the index case: Sputum smear positive TB patients are much more infectious than smear negative patients. Furthermore, the infectivity of smear positive patients is increased as the concentration of the bacilli in their sputum increased (Espinal *et al*., 2000; Peloquin, 2005; Narasimhan, Wood, MacIntyre & Mathai, 2013).

2. Immunity status: The risk for primary active tuberculosis and reactivation of latent tuberculosis is higher in people with immune compromised conditions (Peloquin, 2005; Narasimhan *et al*., 2013). Patients with HIV infection are at high risk to get infected by *M. tuberculosis* and of reactivation of LTB. Furthermore, the disease progression is faster and the mortality rate is higher in HIV-seropositive patients (Braun *et al*., 1991; Peloquin, 2005; Narasimhan *et al*., 2013).

3. Diabetes mellitus (DM): Presence of DM was found to be associated with about threefold increase in the risk of tuberculosis (Jeon & Murray, 2008; Dooley & Chaisson, 2009).

4. Nutrition: Malnutrition status has also been linked to increased risk of tuberculosis, because of its profound effects on the immune system (Chandra, 1997; Cegielski & McMurray 2004; Lonnroth, Williams, Cegielski & Dye, 2010).

5. Age: Children are at increased risk of getting the infection when they are in contact with infectious cases. As much as 80% of children might get the disease when they have prolonged close contact with smear positive patients (Marais *et al*., 2004; Narasimhan *et al*., 2013).

6. Tobacco smoke: The positive relationship between tobacco smoking and the increased incidence of TB infection and TB disease was established in a number of systemic reviews (Maurya, Vijayan & Shah, 2002; Pai *et al*., 2007; Bates *et al*., 2007). For instance, Bates *et al.,* (2007) showed that the relative risk of TB infection was 1.73 higher in smokers, which indicate that the change of TB infection is increased by 1.73 times in smokers. Furthermore, the relative risk of TB disease was between 2.33 to 2.66 times higher in smokers, which indicate that the development of active TB disease is about 2.5 times higher in smokers.

7. Indoor pollution: The use of biomass fuel for cooking is associated with higher risk of the development of TB disease (Mishra, Retherford & Smith, 1999; Kolappan & Subramani, 2009; Pokhrel *et al*., 2010). The mechanism by which biomass fuel could increase the risk of TB is not fully understood; however, animal studies have indicated that wood smoke leads to impairment of macrophage phagocytic function, surface adherence (Zelikoff, *et al.*, 2002) Additionally, biomass combustion leads to release large particulate matter (PM) such as carbon monoxide

(CO), nitrogen oxide, formaldehyde, and polyaromatic hydrocarbons which can deposit deep into the alveoli and destroy them (Boman, Forsberg, & Järvholm, 2003).

8. Alcohol: Alcohol drinkers are at higher risk of TB disease compared with non-alcoholic. The relative risk for TB disease was considerably higher among people who consume more than 40 gram of alcohol per day or who have alcohol use disorder (RR = 2.94 ,95% CI = 1.89– 4.59). (Lonnroth, Williams, Stadlin, Jaramillo & Dye, 2008). Heavy alcohol consumption deteriorates the immune system, specifically the production of cytokine which may explain the increased risk of TB disease in heavy alcohol drinkers (Szabo, 1997).

1.4 Signs, symptoms, and diagnosis of tuberculosis

People with LTB are often asymptomatic but show a positive reaction to the tuberculin test (Peloquin, 2005; Knechel, 2009). Patients with active TB disease typically have symptoms related to the site of the disease. The typical symptoms of pulmonary tuberculosis include; a chronic cough commonly with sputum, which is sometimes blood-stained, dyspnea, and chest pain. Systemic symptoms that might accompany any form of TB include fever, pallor, night sweating, loss of appetite, and loss of weight (Peloquin, 2005; Barker, 2008; Knechel, 2009).

The diagnosis of TB is based on the suggestive symptoms such as a cough or fever for more than two weeks, and loss of weight. The diagnosis of PTB is confirmed by direct sputum smear for acid-fast bacilli (AFB). This test is highly recommended by the WHO because it is the cheapest method that can identify the most infectious cases. Three specimens should be collected to confirm the diagnosis of PTB (WHO, 2010). About 80% of patients show a positive result in the first

sputum specimen; 15% and 5% of patients show a positive result in the second and third sputum specimens respectively (Barker, 2008). The negative sputum smear result doesn't exclude PTB because more than 40% of patients with culture-positive PTB show a negative result with direct smear AFB (Barker, 2008).

The chest radiograph is a sensitive but non-specific tool for diagnosis of pulmonary tuberculosis (Barker, 2008). Unequivocal diagnosis can only be accomplished by culturing the microorganism from the sputum or other biological specimens. Culturing the isolated bacilli is also crucial to determine the sensitivity of the isolated organism to the antituberculosis drugs (Barker, 2008).

1.5 Treatment of tuberculosis

1.5.1 Beginning the chemotherapy of tuberculosis

The era of chemotherapy of tuberculosis was started by the discovery of streptomycin (SM) in 1943 by Albert Schatz and Selman Waksman. SM was the first antibiotic to show activity against *Mycobacterium tuberculosis* with an acceptable level of toxicity (Schatz, Bugle & Waksman, 1944). Although treatment with SM alone reduced the mortality of tuberculosis compared with bed rest, the development of resistance to SM reduced the long-term benefits of SM (Fox, Ellard, & Mitchison, 1999). Soon after the discovery of SM, Jorgen Lehman, a Swedish physician, and chemist, synthesized Para-aminosalicylic acid (PAS) in 1944. PAS has an advantage over SM of being administered orally. PAS was found to be slightly less effective than SM, but its addition to SM significantly improves the activity of SM, and dramatically reduces the emergence of SM-resistant mutants. It was the first time that the importance of combination therapy in preventing the

development of acquired drug resistance was recognized (Fox, Ellard, & Mitchison, 1999).

The great success in the history of TB treatment was made in 1952 by the discovery of the antituberculosis properties of isoniazid (INH) by Gerhard Domagk. INH alone was comparable in its efficacy to a combination of SM and PAS (Medical Research Council, 1952). However, INH- resistance was developed in a high proportion of the patients. Combination therapy with INH and SM significantly reduced the development of INH resistance (Medical Research Council, 1953).

Because of the side effects of PAS, about 15% of the patients defaulted the standard regimen of INH, SM, and PAS (Mount & Ferebee, 1954). For this reason, the search for a more tolerable alternative for PAS was started. The efficacy of ethambutol (EMB) which was introduced in 1962 was compared with PAS. When used in a dose of 12.50 to 25.0 mg/ Kg, EMB was found to be as effective as PAS. Furthermore, substitution of PAS by EMB could also reduce the treatment period from 24 to 18 months (Ferebee, Doster, & Murray, 1966; Bobrowitz & Robins, 1967; Doster, Murray, Newman, & Woolpert, 1973).

The second major advancement in the fight against TB was made through the introduction of rifampicin (rifampin, RIF) in 1963. The addition of RIF to a regimen consists of SM, INH, and EMB could cure more than 95% of the patients in only nine months (Fox, Ellard, & Mitchison, 1999; Iseman, 2002).With the availability of RIF and pyrazinamide (PZA), and recognition of their sterilizing activity, successful treatment of PTB become possible in just 6 months. This regimen is known as short-

course chemotherapy (SCC) (Tripathy, 1982; Iseman, 2002; Zumla, Nahid, & Cole, 2013).

1.5.2 Current treatment of pulmonary tuberculosis patients

For new pulmonary tuberculosis patients, WHO highly recommends the use of 6 months RIF-based regimen (WHO, 2010). This regimen consists of INH, RIF, PZA, and EMB for two months (2HRZE) as the intensive phase, followed by INH and RIF for four months (4HR), as the maintenance phase. Usage of RIF in the intensive phase only is highly discouraged because it was associated with more relapse and death (Menzies *et al*., 2009). RIF is the most powerful sterilizing agent in the SCC. RIF continue to exert its sterilizing effect during the whole course of chemotherapy by killing the semi-dormant population of *M. tuberculosis* which have spurt of active metabolism. If RIF is included in the maintenance phase, this population of *M. tuberculosis* which cannot be killed by other drugs including INH, will continue growing and eventually lead to disease relapse (Mitchison, 1979, Mitchison, 1985). The dosages of the first-line antituberculosis agents are listed in Table 1.1.

Daily administration of antituberculosis drugs during the whole course of treatment is the optimal way and should be applied whenever possible (WHO, 2010). However, when this approach is impractical, the drugs may be given daily in the intensive phase and intermittently during the maintenance phase, provided that, every dose is directly observed (WHO, 2010). The third option is to give the drugs three times weekly during the whole period, provided that, all the doses are directly observed, and the patient is HIV-negative and not living in an HIV-prevalent area

(WHO, 2010). However, acquired drug resistant was significantly higher in new HIV-negative PTB patients who received three times weekly dosing throughout the therapy (Menzies *et al*., 2009). Furthermore, In HIV-positive patients, failure and relapse rate was significantly higher in patients who received intermittent intensive phase (Khan *et al*., 2010).

EMB may be discontinued during the intensive phase if the isolated *M. tuberculosis* was susceptible to INH, RIF, and PZA (Peloquin, 2005). On the other hand, EMB may be added to the continuation phase, in the area where INH resistance is high (WHO, 2010). EMB is thought to protect RIF and prevent the development of multi-drug resistant (MDR) strains of *M. tuberculosis* (Mitchison, 1979).

Table 1.1 Standard doses of the first-line antituberculosis agents

Drugs	Route of administration	Recommended dose			
		Daily		3 times per week	
		Dose (range) mg / kg body weight	Maximum dose in mg.	Dose (range) mg / kg body weight	Maximum dose in mg.
INH	Orally	5 (4 - 6)	300	10 (8 - 12)	900
RIF	Orally	10 (8 - 12)	600	10 (8 - 12)	600
PZA	Orally	25 (20 -30)	2000	35 (30 - 40)	3000
EMB	Orally	15 (15 - 20)	1600	30 (25 - 35)	2400
SM	Intramuscular	15 (12 - 18)	1000	15 (12 - 18)	1500

Adapted from WHO, Treatment of Tuberculosis Guidelines Fourth Edition (WHO, 2010)

1.6 Role of individual drug in the short-course chemotherapy of tuberculosis

1.6.1 Prevention of drug resistance

Due to the large bacterial population present in patients with active PTB, naturally-occurring mutant bacilli resistant to a single drug are usually present (Peloquin, 2005). An addition of one or more drugs is essential to kill those bacilli and prevent them from growing over the sensitive ones (Mitchison, 1979; Peloquin 2005). The efficacy of preventing the emergence of resistant strains to a specific drug depends on the ability of the companion drug(s) to exert a strong and sustainable bactericidal activity (Mitchison 1979; Mitchison, 1985).

The relative efficacy of antituberculosis agents in this regard can be inferred from their prevention of treatment failure due to the development of drug-resistant during the treatment. RIF and SM were found to be more effective than EMB, which in turns was much more effective than PAS, thiacetazone (TB1) or PZA in preventing the emergence of INH-resistant strains (Velu *et al*., 1964; Mitchison, 1979).

INH is superior to SM in preventing the drug-resistance. Treatment failure occurred in 74 % of patients who received TB1 plus SM, and in 28% of patients who received TB1 plus INH regimen (Briggs *et al*., 1968). The antituberculosis agents can be ordered according to their activity in preventing the drug resistance as the follows: INH is the most effective one, followed by RIF and SM. EMB is less effective, but still much better than PZA (Mitchison, 1979; Mitchison 1985)

1.6.2 Sterilizing activity

The sterilizing activity is defined as the speed with which the last viable bacilli are eradicated (Mitchison, 1985). As a result, incorporation of drugs with high sterilizing activity in the treatment regimen will lead to a fast elimination of the bacilli and shortening of the treatment period (Mitchison 1979; Mitchison, 1985). In clinical studies, the sterilizing activity of a given regimen is measured by the rate of sputum culture conversion to negative after two months of therapy, or the rate of relapse after the drugs have been discontinued (Mitchison, 1979; Tripathy, 1982; Mitchison, 1985). In clinical trials, the addition of PZA or RIF to regimen consisted of SM and INH, significantly increased the 2-months culture conversion rate, and decreased the relapse rate, whereas the addition of TB1 did not do so. Therefore, both RIF and PZA have high sterilizing effects but TB1 does not (Fox, Ellard, &Mitchison 1999). The addition of SM to a regimen of INH and RIF slightly but not significantly reduced the relapse rate and increased the 2-months culture conversion rate. Thus, SM appears to have very little sterilizing activity (Mitchison, 1979; Mitchison, 1985; Mitchison, 2000). While both RIF and PZA have the highest sterilizing activity, it was clear that RIF exerts its activity during the whole course of treatment while the activity of PZA diminished after the first two months (Fox, Ellard, &Mitchison 1999).

Replacing PZA by EMB significantly increased the relapse rate and decreased the 2-months culture conversion rate, which indicated the poor sterilizing activity of EMB (Fox, Ellard, &Mitchison 1999). In conclusion, RIF and PZA are the main sterilizing drugs; SM has a moderate sterilizing activity while EMB has no sterilizing activity (Mitchison, 1979, Mitchison, 1985, Mitchison, 2000)

1.6.3 Bactericidal activity

The inherent bactericidal activity of any antituberculosis drug or regimen is best estimated by the early bactericidal activity during the first two days of treatment (EBA_{0-2}) (Mitchison, 1979). The early bactericidal activity was measured as the fall in the counts of colony forming unit (CFU) of patients' sputum at different interval of the treatment. The first study of this kind was conducted in Nairobi by Dr. Amina Jindani and colleagues (Jindani, Aber, Edwards, & Mitchison, 1980; Jindani, Dore, & Mitchison, 2003). Following this study, a number of similar studies were conducted in different countries; these studies have been reviewed by Donald and Diacon (Donald & Diacon, 2008). The results of all EBA studies can be summarized in the following finding; 1) Mycobacterium tuberculosis is killed much more rapidly during the first two days of the treatment; 2) bactericidal activity of INH is much higher than that of any other single agent; 3) the EBA activity of any agent is increased considerably by addition of INH; 4) the high bactericidal activity of INH is dramatically reduced after the first two days; 5) RIF has much less bactericidal activity than INH in the initial two days, but it maintains its activity during the latter period; 6) the high sterilizing activity of RIF and PZA cannot be explained on the basis of their bactericidal activity since both drugs have modest bactericidal activity (Mitchison, 1979; Mitchison, 2000; Donald, & Diacon, 2008).

1.7 Basic pharmacology of INH, RIF, and PZA

1.7.1 Basic pharmacology of INH

After oral administration, INH is rapidly and completely absorbed and reaches its C_{max} within 1- 2 hours when taken on an empty stomach (Douglas, & McLeord, 1999; Peloquin 2002; Budha, Lee, & Meibohm, 2008). Food, especially high-fat meals decrease and delay its absorption, leading to a reduction of its C_{max} almost to the half, and doubling of its T_{max} (Lin, Lin, Chan, & Lu, 2010). It is metabolized in the liver by acetylation and dehydrazination. The acetylation pathway is mediated by N-acetyl transferase 2 (NAT2) (Budha, Lee, & Meibohm, 2008). The patients are either slow acetylator or rapid acetylator. The serum half-life ($t_{1/2}$) of INH is about 1.5 hours in rapid acetylator and about 4 hours in slow acetylator. Most Asians and about half of Caucasian and Blacks are rapid acetylator (Douglas, & McLeord, 1999; Budha, Lee, & Meibohm, 2008).

INH enters tubercle bacilli by passive diffusion and gets activated by *M. tuberculosis* catalase-peroxidase enzyme (KatG), which is encoded by the *katG* gene (Shi, Itagaki, & Sugawara, 2007; Budha, Lee, & Meibohm, 2008). This activation leads to a production of a wide variety of compounds includes; isonicotinic acyl-NADH, an enoyl carrier protein reductase “InhA” inhibitor, reactive oxygen species, hydroxyl radicals and reactive organic species. These reactive species and radicals affect the *M. tuberculosis* at multiple cellular targets and eventually lead to bacterial cell death (Lei, Wei, & Tu, 2000; Shi, Itagaki, & Sugawara, 2007). Inhibition of InhA enzyme that catalyzes the final step in elongation of mycolic acid is the most important mechanism by which INH kills the *M. tuberculosis* (Vilchèze *et al*., 2000).

Adverse effects of INH are dose-related. At the typical doses of 3-5 mg/kg, 1 to 5 % of patients experience some of its adverse effects. At a dose of 10 mg/kg, the adverse effects may occur in 15 % of the patients (Goldman & Braman, 1972). Peripheral neuropathy is the commonest toxic effect of INH. It occurs as a result of increased pyridoxine excretion, leading to pyridoxine deficiency. It is especially prevalent in alcoholism, malnutrition, and slow acetylator (David & Leahy, 1959; Goldman & Braman, 1972). INH-induced peripheral neuropathy is preventable and reversible by administration of pyridoxine (Goldman & Braman, 1972). Central nerves system adverse effects include; headache, irritability, restlessness, lethargy, drowsiness, seizure, and encephalopathy. Depression and other psychological effects such as unbalanced state of mind, impaired memory were reported during INH therapy, especially in alcoholism, and in patients with a history of mental illness (Goldman & Braman, 1972; Cheung, Lo, Lo, Ip, & Cheng, 1993).

Clinical hepatitis induced by INH is a very severe side effect that occurs in 1% of the patients and requires immediate drug discontinuation. It is manifested by loss of appetite, nausea, vomiting, jaundice, and right upper quadrant pain. In most cases, INH-induced hepatitis occurs three months after the initiation of drug therapy, but it might occur after one week in some patients (Goldman & Braman, 1972; Tostmann *et al*., 2008). The risk of INH-induced hepatitis is higher in alcoholics, during pregnancy, and with advanced age (Thompson *et al*., 1995).

1.7.2 Basic pharmacology of RIF

RIF is well absorbed after oral administration, and reaches its maximum concentration after 2 - 4 hours (Douglas, & McLeord, 1999; Peloquin 2002; Budha, Lee, & Meibohm, 2008). The food decreases and delays its absorption, leading to a

significant reduction in its C_{max} and considerable increases in its T_{max} (Lin, Lin, Chan, & Lu, 2010). RIF is metabolized in the liver to 25-desacytyl RIF, which retains most of the RIF activity. Owing to its potent enzyme induction property, RIF induces its own metabolism (auto-induction). The serum half-life of RIF is reduced by about 40% in the first two weeks of its administration as a result of its auto-induction property (Benedetti, & Dostert, 1994).

RIF acts by binding to and inhibiting DNA-dependent RNA polymerase, the binding of RIF occurs at the β subunit of this enzyme leading to blocking of the elongation process of the growing RNA. Most of the resistance to RIF occurs as a result of a mutation in this β subunit gene (*rpo B*) (Hartmann, Honikel, Knusel, & Nuesch, 1967; Shi, Itagaki, & Sugawara, 2007; Budha, Lee, & Meibohm, 2008).

Hepatotoxicity is the most serious adverse effect of RIF. When RIF was used alone as a preventive therapy for patients who cannot tolerate INH, the incidence of hepatotoxicity was found to be in the range of 1 - 2 % (Villarino *et al*., 1997; Tostmann *et al*., 2008). When RIF was given with non-hepatotoxic drugs (EMB or SM) the incidence of hepatotoxicity was about 1.1% (Steele, Burk, & DesPrez, 1991). RIF significantly increases the hepatotoxicity of INH, probably due to increasing INH metabolism and the production of its hepatotoxic metabolites (Steele, Burk, & DesPrez, 1991; Tostmann *et al*., 2008).The mechanism by which RIF causes hepatotoxic effect is not clear, nor predictable. However, it seems to involve hypersensitivity reaction and appears to be dose dependent, and more frequent with large intermediate dosage (Martinez, Collazos, & Mayo, 1999; Saukkonen *et al*., 2006; Tostmann *et al*., 2008). RIF may cause a flu-like syndrome,

especially if given less frequently than twice weekly (Addington, 1979; Chan-Tompkins, 1995).

1.7.3 Basic pharmacology of PZA

PZA is readily absorbed after oral administration, reaching its maximum concentration after 1 to 2 hours (Peloquin 2002; Budha, Lee, & Meibohm, 2008). The food and antacid slightly reduce the C_{max} of PZA (Lin, Lin, Chan, & Lu, 2010). PZA is hydrolyzed by a microsomal deaminase to the active metabolite, the pyrazinoic acid (POA). Pyrazinoic acid is then hydroxylated by xanthine oxidase to 5-hydroxypyrazoinoic acid. The serum half-life of PZA is about nine hours (Budha, Lee, & Meibohm, 2008).

Despite its importance in the treatment of tuberculosis, the mechanism of action of PZA is still not fully understood. PZA is a prodrug, it is converted to its active form pyrazinoic acid (POA) by bacterial nicotinamidase/pyrazinamidase (PZase) which is encoded by the gene pncA (Zhang, & Mitchison, 2003). POA disrupts the membrane energetics and inhibits membrane transport function in *Mycobacterium tuberculosis* (Zhang, Wade, Scorpio, Zhang, & Sun, 2003). Resistance to PZA occurs due to inactivation of (PZase) enzyme as a result of a mutation in *pncA* gene (Zhang, Wade, Scorpio, Zhang, & Sun, 2003; Zhang, & Mitchison, 2003).

The activity of PZA depends on the pH of the medium. It shows no activity under normal culture condition, but shows high activity in acidic condition. The activity of PZA in acidic medium makes it ideal for killing the bacilli that live in acidic phagosomes inside the infected macrophages. The activity of PZA against this

bacterial population increases as the growth rate of this organism decline (Mitchison, 1979).

Hyperuricemia and non-gouty polyarthralgia are the most common side effects of PZA. Half of the patients may experience an asymptomatic elevation of serum uric acid, due to the inhibition of urinary excretion of uric acid (Zierski, & Bek, 1980; Steele, & Des Prez, 1988). The incidence of symptomatic arthralgia ranges from 1% to 7% depends on the dose of PZA and frequency of its administration (Jenner *et al*., 1981). Interestingly, treatment with RIF has been shown to decrease the incidence of PZA-induced arthralgia, probably due to the enhancement of uric acid excretion and decrease of its deposition in the joints (Sarma *et al*., 1983). Hepatotoxicity is the major serious adverse effect of PZA. With the currently recommended dose of 20 - 30 mg/Kg, the incidence of PZA-induced hepatotoxicity is about 2%, which is similar to INH or RIF-induced hepatitis (Yee *et al*., 2003).

1.8 Problem statements

First-line antituberculosis agents (INH, RIF, and PZA) are administered in doses based on patients' body weight, which is the only factor that has been considered in calculating the dose (WHO, 2010). In most cases, the recommended weight-adjusted doses of these agents are appropriate and lead to successful treatment. However, in some cases, the treatment failure may be due to inappropriate doses, and therefore individualizing the drug doses for those patients could improve their treatments outcomes (Yew, 2001; Peloquin, 2002). Individualizing the drugs' doses can be guided by the use of therapeutic drug monitoring (TDM). TDM can be simply defined, as measuring the concentration of

a drug in the biological fluid, and adjustment of its dose accordingly. The purpose of TDM is to achieve the desirable drug level that leads to maximum efficacy and minimum toxicity (Kang, & Lee, 2009).

For any drug to be a candidate for TDM, the following criteria should be met: 1) Good correlation between the drug exposure and its clinical efficacy or toxicity or both must be established. 2) A definite therapeutic range has been set. 3) Significant pharmacokinetic variability is expected among the patients (Kang, & Lee, 2009).

In the case of INH, RIF, and PZA some of these criteria are not yet met. This issue will be discussed in more details in the next chapter. In summary, the following reasons are precluding the routine use of TDM to guide the dosing of these antituberculosis agents:

1. Further evidence is required for better judgment of the relationship between drug exposure and clinical efficacy as it remains contrary.
2. A definite therapeutic range has not been established for INH, RIF, and PZA. The C_{max} is the target for TDM because their trough concentration is usually below the detection limit and of no clinical value (Peloquin, 2002). However, there are two major problems with the utility of the C_{max} of these agents. Firstly the exact ranges of the C_{max} of these agents are not well-defined. Secondly, the time to reach the C_{max} (T_{max}) varies from drug to another and from patient to patient regarding the same drug.
3. There is some contradictory about the factors that determine the C_{max} of INH, RIF, and PZA, such as the effect of patient's body weight, the patient's age, the

patient's gender, and the presences of other disease. This issue will be discussed in details in the next chapter.

1.9 Rational of the study

INH, RIF, and PZA are essential drugs in the treatment of tuberculosis. These agents are administered in fixed-doses based on the patient's body weight. Although WHO guideline for management of tuberculosis (WHO, 2010) stated a maximum dose of each drug that should not be exceeded, some authors don't agree with the use of term "maximum dose" and believe that, the maximum dose of a particular drug for a particular patient is the highest dose of that drug that the patient can tolerate, with an acceptable benefit/risk ratio (Peloquin, 2002).

This study is designed to test the concentration-response relationship of INH, RIF, and PZA. The outcomes being explored are the treatment success rate and development of adverse drug reaction. The independent variables of interest are the plasma concentration of INH, RIF, and PZA. If the study could establish a significant association between the concentration of any one of these drugs and patients' response or development of drug side effects, it would be rational to use the therapeutic drug monitoring (TDM) to guide antituberculosis therapy. The second main objective of this study is to find out the patients' demographic and clinical factors that may affect the plasma concentration of these drugs. Identifying those patients will allow the physician to pay more care to them which will lead to more successful management.

1.10 Aim of the study

To obtain a baseline pharmacokinetic data concerning the blood concentration of INH, RIF, and PZA in Malaysian tuberculosis patients and to assess the influence of these pharmacokinetics data on the treatment of pulmonary tuberculosis patients

1.10.1 Specific objectives of the study

1. To develop and validate high-performance liquid chromatography methods for determination of INH, PZA and RIF in human plasma.

2. To measure the plasma concentrations of INH, RIF, and PZA in a cohort of Malaysian tuberculosis patients.

3. To assess the effect of the pharmacokinetics of INH, RIF and PZA on the treatment outcomes of PTB patients and the occurrence of drug adverse effects.

4. To find out the clinical and social factors with significant effects on the plasma concentration of INH, RIF, and PZA.

CHAPTER 2

LITERATURE REVIEW

2.0. Pharmacokinetics and pharmacodynamics of INH, RIF, and PZA

In general, antibacterial agents are either bactericidal or bacteriostatic. INH, RIF, and PZA are all bactericidal drugs (Nuermberger & Grosset, 2004). The bactericidal drugs can be divided into two categories based on the pattern of their bactericidal activity. The first category is the concentration-dependent bactericidal agents, for which the rate of killing is increased by increasing the concentration of the drug at the site of infection. For these antibacterial agents, the rate of bacterial killing is best correlated with the C_{max}/MIC or the AUC/MIC ratio (Craig, 1998). To achieve the highest bactericidal activity of these antibiotics, they should be given at the largest safe dose that yields the highest possible concentration of the drug at the site of infection (Craig, 1998; Nuermberger & Grosset, 2004).

Animal studies and *in vitro* studies indicate that INH, RIF, and PZA are bactericidal drugs with concentration-dependent activity. In a murine model of aerosol infection, Jayaram *et al*., (2003) demonstrated that the bactericidal activity of RIF is best correlated with its AUC/MIC ratio (r^2 = 0.95), followed by its C_{max}/MIC ratio (r^2 = 0.86). In another experiment on the same model, Jayaram *et al*., (2004) found that INH is exhibiting a concentration-dependent bactericidal effect on extracellular *M. tuberculosis*. This bactericidal activity was related to $AUC_{0\text{-}24h}$ / MIC ratio and was not related to the duration of exposure.

Gumbo *et al*., (2009) showed that the sterilizing activity of PZA was related to its $AUC_{0\text{-}24h}$/ MIC ratio and 90% of the maximal effect was achieved when this ratio was equal to 209. In contrast, the ability of PZA to suppress the emergence of bacterial resistance was best correlated with the duration of time in which the concentration of PZA remains above its MIC (T_{MIC}). This observation indicates that both the dose size and administration schedule are important for PZA to exhibit its sterilizing and resistance-suppression activities.

2.1 Literature search

The following databases; PubMed, EMBASE, Cochrane Library, were searched for the studies that were published in English and was investigating any of the following issues; 1) the pattern of INH, RIF or PZA concentration in the blood, 2) factors affecting the concentration of INH, RIF, or PZA in TB patients or healthy volunteers, 3) the relationship between the concentration and activity of INH, RIF, and PZA, 4) the relationship between the concentration of INH, RIF or PZA and the development of adverse drug reaction (ADR). The keyword used for the search were 'concentration' or 'level' or 'peak' AND 'antituberculosis' or 'antimycobacterial' or isoniazid 'INH' or 'rifampicin' (RIF) or 'pyrazinamide' (PZA) AND 'patients response' or patients outcome' or development/incidence of adverse drug reaction/side effects or AND 'factor affecting' or 'determinants'. Only human studies that include adult tuberculosis patients or adult healthy volunteer were included. Any study design such as randomized clinical trial, prospective study, retrospective cohort study, case-control study, was accepted. Review articles and meta-analysis studies were also considered for the review purpose.

The studies identified and accepted for this literature review can be divided into three groups

1. Explanatory studies which measure the pharmacokinetic parameters of INH, RIF or PZA and correlate them with the treatment outcomes or development of ADR. The subjects of such study must be adults with PTB with or without other forms of EPTB.

2. Explanatory studies which measure the pharmacokinetic parameters of antituberculosis agents and address the factors that affect those pharmacokinetic parameters. The subject of these studies may include adult healthy volunteers, or adult TB patients with or without other comorbidities, or both.

3. Observatory studies that measure the pharmacokinetics of INH, RIF, and PZA without explaining the variability in those pharmacokinetic parameters or correlating the pharmacokinetic parameters with the treatment success or development of ADRs.

2.1.1 The relationship between the pharmacokinetics of INH, RIF, and PZA and the treatment outcomes of PTB patients

Gangadharam *et al*., (1961) measured the serum concentration of INH in sub-group of patients who participated in clinical trials in India (Tuberculosis Chemotherapy Centre, 1960). The patients were divided into four groups to receive one of the following regimens; 400 mg of INH once a day (HI-1), 200 mg of INH twice a day (HI-2), 200 mg of INH once a day (H), or 100 mg of INH plus PAS 5 mg twice a day (HP). The serum concentration of INH was higher in patients who received 400 mg of INH in a single dose than the patients who received 200 mg of INH twice daily. For slow-acetylator, the C_{max} of INH was 6.6 mg/L and 2.6 mg/L in