International Journal of Medicine and Pharmacy
December 2014, Vol. 2, No. 2, pp. 45-59
ISSN 2372-5087 (Print) 2372-5095 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/ijmp.v2n2a4
URL: http://dx.doi.org/10.15640/ijmp.v2n2a4

Comparative Pharmacokinetics of Kanamycin between Multi-drug Resistant Tuberculosis Patients and Healthy Volunteers

Thinn Yu Aung[1], May Hla Thwin[2], Thaw Zin[1], Khin Nwe Oo[3] & Nu Nu Aye[2] & Shwe Sin[1]

Abstract

Emergence of MDR-TB has become a global emergency and significant public health problem worldwide. The main problem of second-line anti-TB drugs are high cost, lower efficacy, greater toxicity and prolong treatment required, resulting in low compliance, high defaulter, low sputum conversion and high relapse rate. With DOTS-Plus pilot project being implemented in Myanmar, there is a need to ensure good practice of reserve drugs like Kanamycin, used extensively by the National TB Program, from becoming resistant. Since TB control required an integrated approach, the study explored the socio-demographic, clinical and pharmacokinetic factors that can influence the outcome of treatment. A total of 20 healthy volunteers and 20 MDR-TB patients were recruited for pharmacokinetic study and Kanamycin levels analyzed by bioassay using *Bacillus subtilis*. The study showed that majority of the MDR-TB recruited were from low education, low income working males with family history of TB contact. Bad habits like smoking, delay in taking proper treatment and poor compliance to DOTS regime was seen in 60% of patients. Comparison with healthy volunteers indicated that MDR-TB patients, although having lower body weight but no significant difference in pharmacokinetic profile, either in bioavailability (AUC; 80.3±35.9vs73.5±26.2μg/hr.ml), distribution (Vd; 49.1±23.1vs46.3±20.2 liters) or elimination (T1/2; 2.3±0.5vs2.1±0.8 hr). Drug levels were well above MIC. All patients tolerate well to treatment. Follow-up for one year showed significant improvement in CXR with 60%of patients having sputum conversion to negativity within 6 months of treatment. This indicated Kanamycin as a safe and effective drug for use in MDR-TB in Myanmar.

Keywords: Multi-drug Resistant Tuberculosis (MDR-TB), Kanamycin, Pharmacokinetics, Adverse effects

[1] Department of Preclinical Science, Universiti Tunku Abdul Rahman
[2] Department of Pharmacology, University of Medicine (2), Yangon
[3] Department of Medical Research, (Lower Myanmar)

1. Introduction

Drug resistance in tuberculosis is global emergency. Emergence of MDR-TB has become a global emergency and significant public health problem worldwide. The main reasons for the increasing burden of TB globally are poverty and the widening gap between rich and poor in various populations in developing countries, disenfranchised urban populations in developed countries, neglect of the disease (inadequate case detection, diagnosis and cure) collapse of the health infrastructure in countries experiencing severe economic crisis or civil unrest and the impact of the HIV pandemic worldwide [1].

The main problem of second-line drugs used for MDR-TB is high cost, lower efficacy, greater toxicity and prolong treatment required, resulting in low compliance, high defaulter, low sputum conversion and high relapse rate. Aminoglycosides remains the mainstay in the treatment of MDR-TB and all regimes currently used in Myanmar which includes kanamycin injection [2, 3].

2. Objective

2.1 General Objective

To compare the pharmacokinetics of Kanamycin in between MDR-TB patients and normal healthy volunteers

2.2 Specific Objectives

1. To validate the bioassay method for determination of serum kanamycin
2. To compare the pharmacokinetics parameters of Kanamycin in MDR-TB patients and healthy volunteers given by intramuscular route
3. To find out the response in MDR-TB patients of Union Tuberculosis Institute (UTI) Yangon taking treatment regimen on kanamycin
4. To explore the social demographic factors likely to contribute treatment failure in MDR- TB patients at the Union Tuberculosis Institute (UTI), Yangon

3. Materials and Methods

Hospital-based, analytical study, using controlled parallel-group design was carried out on 20 healthy volunteers and 20 MDR-TB patients attending Union Tuberculosis Institute (UTI) Yangon/Aung San from January 2011 to September 2011.

In this study, agar disc diffusion bioassay method was used for determination of serum kanamycin concentration. Considerably better sensitivity and specificity have been achieved by high performance liquid chromatography with UV detection but it is neither economically nor technically feasible for routine assessment in most laboratories. Other methods that can use for determination of kanamycin were urease method, long-wavelength fluorimetery method, enzyme immunoassays and radioimmunoassay method.

3.1 Patient Selection

A total of 20 healthy volunteers and 20 patients admitted to/attending the OPD of Aung San TB Hospital and Union Tuberculosis Institute, Aung San, Yangon, Myanmar (Burma) were selected according to the criteria set. Selection was done on days which TB patients came to the TB Clinics to receive their anti-TB drugs according to WHO/DOTS regime. Informed consent was obtained before the study.

3.2 Ethical Consideration

The patients were explained about the detailed procedure and written informed consent was obtained before the assessment of the study. Patients who do not wish to participate or request to withdrawal from the study at any time doing this assay period will be allowed without effecting the current or further treatment. Ethical issue approved by board of University of Medicine 2, Yangon.

3.3 Baseline Examination

A baseline examination consisting of history, clinical examination and laboratory investigations were carried out before entry into the trial to exclude organic disease or dysfunction.

Quantitative assay of drugs in blood samples were carried out at the Bacteriology Department and National Poison Control Center (NPCC), DMR-LM (Department of Medical Research, Lower Myanmar).

3.4 Socio-Demographic and Clinical Assessment

This was done by pre-tested questionnaires and face to face interview composing of patient's socio-demographic characteristics, clinical symptoms, diagnosis of tuberculosis, previous and current treatment, where the patients were asked about the drugs taken, how they were taken, dosage regimens, side effects and adverse drug reaction monitoring, according to WHO guideline. Clinical assessment was done by TB specialists on admission and follow-up assessment was done throughout the study.

3.4 Collection of Blood Samples

Serial blood samples were obtained at (0.5, 0.75, 1, 2, 4, and 6) hours after administration of IM kanamycin 1 gm and the serum harvested and stored at freezer -20°C until assay.

3.5Assay Methodology

3.5.1Preparation of Standards

Kanamycin pure powder from A.N.B Laboratories Co. Ltd, Thailand was taken and weighed 30 mg (contain 25 mg kanamycin base). And then put into a volumetric flask and dissolved in distilled water made up to 25ml. The volumetric flask was shaken to obtain a stock solution of 1 mg/ml. This stock solution was serially diluted to obtain 16, 8, 4, 2, 1 μg/ml

One ml of *Bacillus subtilis* broth was added and poured onto petri dishes. After that 6 small wells were punched down. Six Toyo filter papers (Toyo Roshi Kaisha Co. Ltd., Japan) one with plasma (control) and 5 wells with different concentration of kanamycin 1, 2, 4, 8, 16μg/ml were placed on the 6 punch wells with flame forceps and gently pressed down to ensure contact. The plate was incubated for at 37° C overnight. At the end of the incubation period, the zone of inhibition was measured with a thin plastic transparent ruler and a calibrated curve was drawn accordingly.

3.5.2Validation of Assay Method

Prepartion of Standard Curve

A standard curve was drawn in which a series of stanadards(known concentration of kanamycin 10 μg, 20 , 30, 40, 50, 60 μg) were plotted in the X-axis aginst their measured zone of inhibition (mm) in the Y-axis. The regression line was drawn using the least square method. The standard curve was drwan to check if Beer`s law was obseyed. When the 'r' value of standard curve was less than 0.98, the whole procedure was repeated.

Measurement of Zone Diameters

After the incubation periods, the plate was removed from the incubator and placed in the correct position. Beginning with the top left-hand zone (arrow show in figure 12 and 13), the diameters of the zones of inhibition on the surface of the medium was measured to the nearest 0.1 mm with needle-point calipers.

3.6 Calculation of Parameters

The average values of the diameters of the zones of inhibition of standard and samples were calculated.

The logs of the standard concentrations were plotted on the horizontal axis (x) against the response in terms of the mean diameter of the replicate zones of inhibition on the vertical axis (y) using semi-logarithmic graph paper. The best fitting straight line was constructed by connection the points. (The standard curve of kanamycin was showed in Figure 2). The concentration of the drug in the sample was determined by extrapolation from the standard lines.

For determination of the half life of the drug, the semi-logarithmic graph paper was used. Starting from the peak (maximum) concentration in serum of last sampling time, linear regression was calculated. The results were plotted on semi-logarithmic graph paper. The calculated line was extrapolated back to obtain the concentration of drug in plasma at time zero (C_0). This theoretical concentration is not measured by sampling since mixing of the drug is not instantaneous.

The $t_{1/2}$ or half life is the time taken for the concentration of drug in the blood to decline to half of its original value.

The average volume of distribution (V_d) is obtained by dividing the dose with C_0.

$$V_d = \frac{Dose}{C_o}$$

By simple calculation from $t_{1/2}$, the elimination rate constant k_{el} can be obtained.

$$k_{el} = \frac{0.693}{t_{1/2}}$$

Clearance (Cl) is given by the relationship

$$Cl = \frac{Dose}{AUC}$$

Area under the curve (AUC) can be readily calculated from clearance.

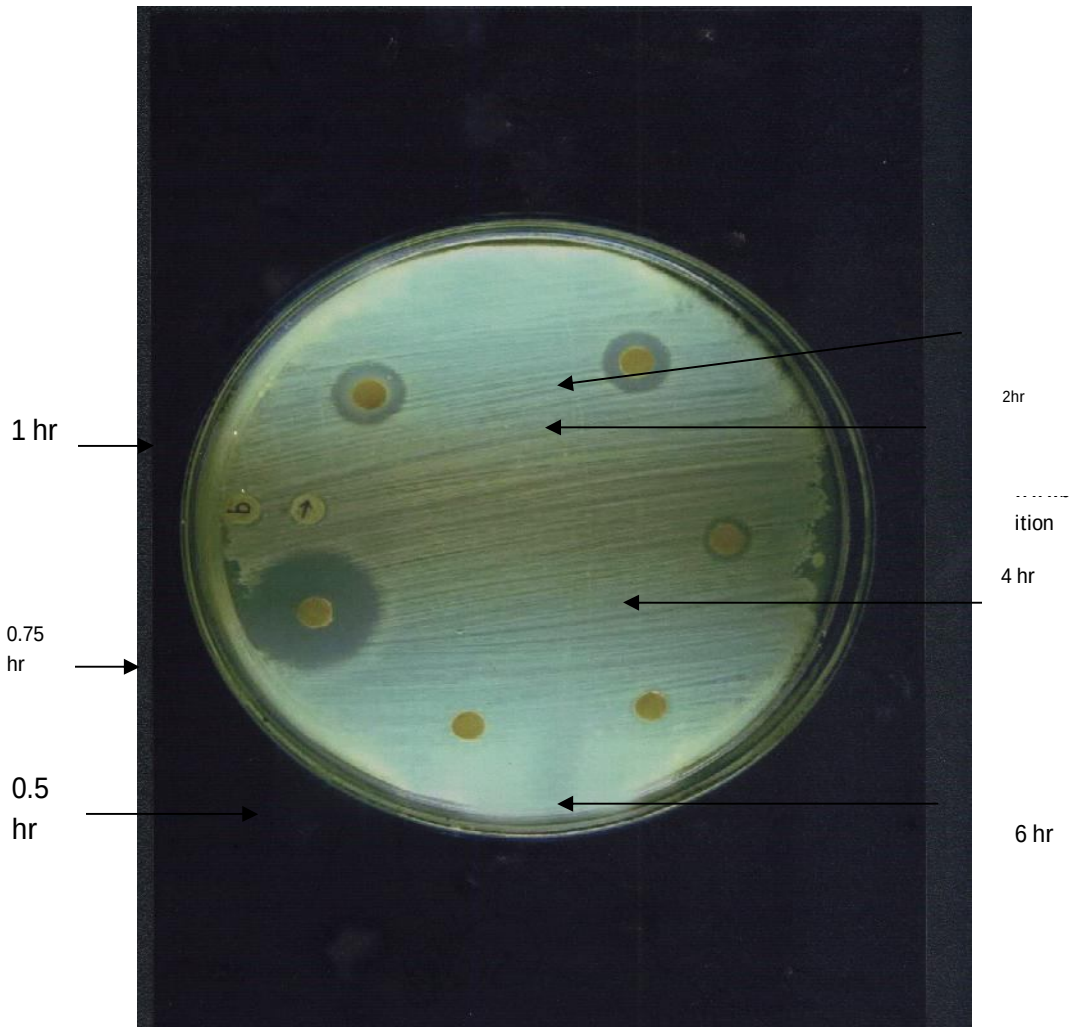

Figure 1. Serum Kanamycin Studied by Blood Agar Disc Diffusion Method

3.7 Follow-Up Assessment

Patients were assessed after 2, 3 and 6 months by sputum AFB and if and when relapse occurred. Individual responses were studied including weight gain and severity as assessed from chest x-rays, sputum AFB, recording of attendance of follow-up, clinical and laboratory improvement and the occurrence of adverse effects assessed weekly. Sputum culture and sensitivity were done on patients showing sputum positive, failure to treatment after 3 months.

3.8 Data Analysis

Serum kanamycin levels were analyzed by agar disc diffusion method using *Bacillus subtilis* (Clarke *et al*., 1969) and pharmacokinetics calculated accordingly using population pharmaco-kinetic software (KINETICA 4.1.). Data entry and analysis will be done by Microsoft Excel 5.0 and curve fitting were done using PRISM 1.0. Continuous data were compared using two-tailed Student's t test, with a 'p' value of 0.05 as the minimal level of significance.

4. Results

4.1 Socio-Demographic Characteristics

The socio-demographic characteristics of MDR-TB patients and healthy volunteers are shown in Table (I). They were comparable for age, body weight and education status. Most of the MDR-TB patients were from low income class and 15% were dependent on their family. More than half (60%) gave history of smoking although 40% have given up their habit since the start of treatment. More than 60% gave history of TB contact either at home from family members or at work. All patients were symptomatic with the main presenting symptoms being persistent cough (70%), low grade fever (40%), chest pain (20%), weight loss (20%), breathlessness (15%) and loss of appetite (10%).

Table I: Demographic Data of MDR – TB Patients and Normal Volunteers

Demographic data		MDR – TB patients Number(percentage)	Normal volunteers Number (percentage)
Sex	Male	13 (65%)	12 (60%)
	Female	7 (35%)	8 (40%)
Age	Mean age in year	38.30 ± 12.64	31.40 ± 7.64
Occupation	Working group	13 (65%)	15 (75%)
	Dependent	7 (35%)	5 (25%)
Education status	Primary school	5 (25%)	3 (15%)
	Middle school	8 (40%)	9 (45%)
	High school	2 (10%)	4 (20%)
	University school	5 (25%)	4 (20%)
Smoking history	history of smoking	12 (60%)	10 (50%)
	no smoking history	8 (40%)	10 (50%)
Monthly family income	10,000-40,000kg	10 (50%)	10 (50%)
	40,000-80,000kg	8 (40%)	9 (45%)
	> 80,000	2 (10%)	1 (5%)
History of TB contact	Yes	12 (60%)	-
	No	8 (40%)	-
Signs and Symptoms of TB	Cough	14 (70%)	-
	Low grade fever	8 (40%)	-
	Chest pain	4 (20%)	-
	Weight loss	4 (20%)	-
	Breathlessness	3 (15%)	-
	Loss of appetite	2 (10%)	-
Weight	Weight in kg	49.75±6.21	52.05 ±6.78

4.2 Adverse Effect

The commonest side effects were gastrointestinal, including nausea, vomiting and anorexia. Neurological effects include hearing disturbance which was more common in healthy volunteers (15%). Signs of renal toxicity and electrolyte disturbance were not seen and all patients tolerate well to treatment.

4.3 Comparison of Pharmacokinetics Parameters

Comparison of pharmacokinetics parameters between two groups indicated a higher area under concentration time curve (AUC) in MDR-TB patients, who had a longer elimination half life and a lower peak serum concentration than healthy volunteers but the difference was not statistically significant. Other parameters were more or less comparable (Table II & Fig 1)

Table II. Comparison of Mean Pharmacokinetics Parameters of Kanamycin in Healthy Subjects and MDR-TB Patients (n=20)

Parameters	Units	Healthy volunteers	MDR-TB patients	P value
C_{max}	μg/ml	16.43± 8.04	15.93 ± 7.01	NS
t_{max}	hours	1.32 ± 0.2	1.38 ± 0.17	NS
AUC	μg/ml.hr	73.53 ± 26.24	80.32 ± 35.91	NS
Vd	liters	46.29 ± 20.18	49.12 ± 23.05	NS
Cl	liters/hr	15.11 ± 4.96	15.11± 7.31	NS
$t_{1/2e}$	hours	2.15 ± 0.81	2.31 ± 0.5	NS
Kel	hours $^{-1}$	0.37 ± 0.12	0.31 ± 0.06	NS
$t_{1/2ab}$	hours	0.49 ± 0.09	0.49 ± 0.1	NS
K_{ab}	hours^{-1}	0.46 ± 0.3	0.46 ± 0.3	NS

NS= not significant

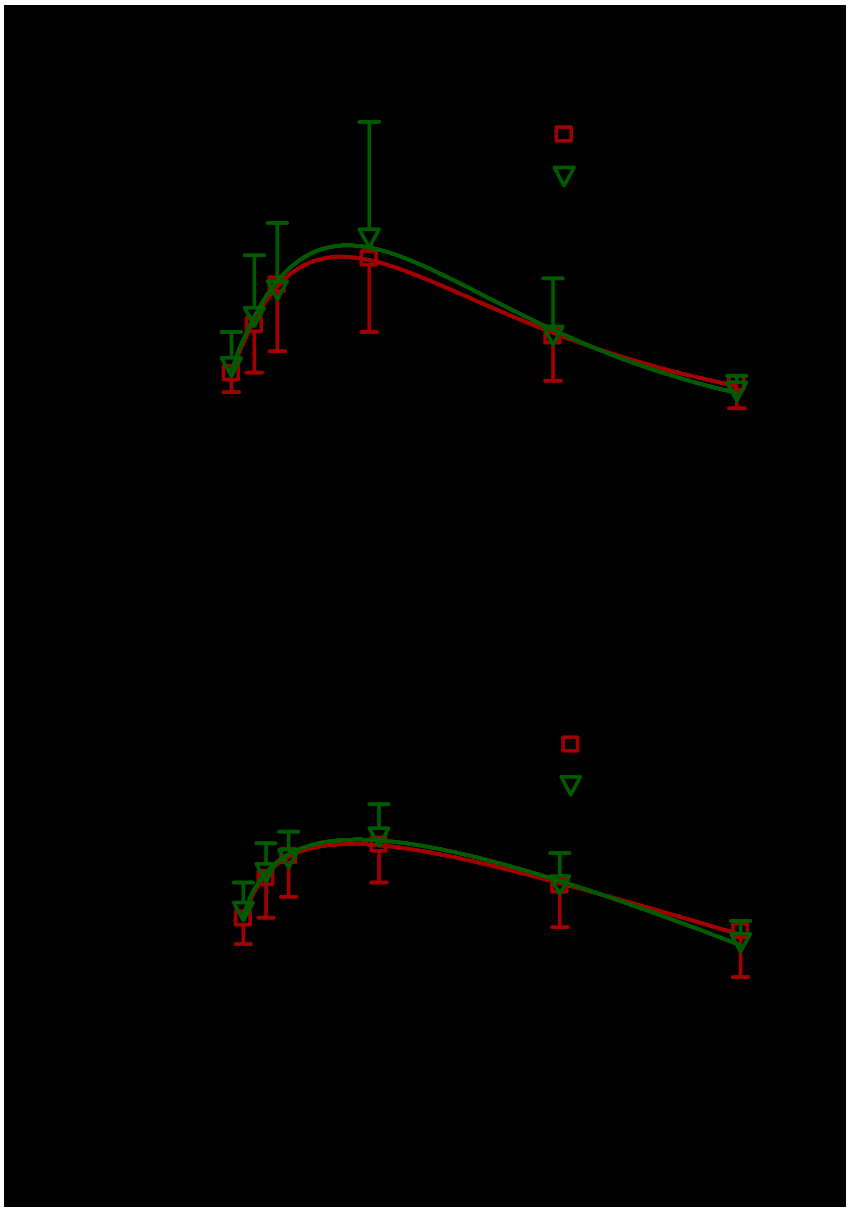

Figure 2. Kanamycin Plasma Concentration Time Curve in Healthy Volunteers and MDR-TB Patients

4.4 Response to Treatment

All patients showed good clinical response after 6 months indicating increase in weight and relief of symptoms such as cough, fever, breathless, haemopytosis and loss of appetite.

Follow-up for one year showed significant improvement in CXR with 5% of patients having sputum conversion to negativity within 3 months and 60% within 6 months of treatment. Four patients still remained smear positive after 1 year of study, and the remaining 4 patients did not complete the treatment.

5. Discussion

The emergence of resistance to drugs used to treat tuberculosis (TB), particularly multi-drug resistant TB (MDR-TB), has become a significant public health problem and the WHO estimates that there were about 450,000 new (incident) MDR-TB cases in the world in 2012. More than one half of these cases occurred in China, India, and the Russian Federation [16]. A DOTS-Plus pilot project has now been implemented in Myanmar since January 2009, at the Aung San TB (ASTB) Hospital and 5 selected townships. With this implementation, there is a need to ensure good practice to prevent potential misuse of reserve drugs as well as false sense of security which might create an untreatable epidemic in future.

5.1 Demographic Characteristics

The WHO has mentioned that a rational approach to management of TB is complex and involves not only the administration of drugs, but also consideration of socio-economic and demographic characteristics that can act as barriers to successful treatment (WHO Report, 2008). The present study showing the age of MDR-TB patients to be 38.3±4.13 years is consistent with WHO report that more than 80% are of productive age with male patients predominating over females in a ratio of 3:1, which was also seen in other studies that males are more likely to have multiple drug resistance [13]. MDR-TB was also found to be most prevalent among low socio economic class, especially working males, married (60%), having large family size (1-12 family number) and (60%) had family contact history of TB that leading to over crowed living conditions acting as contributory conditions for contacting TB which were all well-known contributory factor in TB and drug resistance and failure [8, 7].

5.2 Variability in Serum Kanamycin Concentration

Inter-individual variation was considerable with peak concentration (Cmax) and area under curves (AUC) values, which reflect the amount of kanamycin reaching the systemic circulation [13], although the pharmacokinetics of healthy subjects and MDR-TB patients were not significantly different, as seen in previous studies [14, 15].

AUC values were seen ranging from 41.47-150.18μg/ml.hr in healthy subjects, varying by nearly four folds while Cmax ranged from 8.29-40.5μg/ml, a five fold difference observed in healthy subjects. A greater variation was seen in MDR-TB patients with AUC values ranging from 28.40-170.30μg/ml. hr. and Cmax from 5.57-32.37μg/ml, both varying by nearly six folds. Regarding peak concentration, the lowest kanamycin concentration was μg/ml and the highest concentration was and nearly six folds difference was observed in MDR-TB patients. This is of great importance because the clinical effect of kanamycin is dependent on the concentration of the drug in the serum and such variation may result in suboptimal levels in some subjects and the subsequent selection of resistant strains.

5.3 Adverse Effects

The timely and intensive monitoring and management of adverse effects caused by second-line drugs are essential components of MDR-TB control. The common adverse effect of kanamycin, hearing disturbance (15%), was reported that ototoxicity (hearing loss) was not associated with size or frequency of dosage, but more with older age, longer duration of treatment, and greater total dose received [9,10]. Renal toxicity and electrolyte imbalance not seen in the study is consistent with other studies which indicated that nephrotoxicity was not associated with any patient characteristics or with the size or frequency of dosage. Such nephrotoxicity could not be predicted, but it was generally mild and reversible [9, 10]. Since MDR-TB patients are usually treated with multiple drugs, it is possible that the adverse events could be caused by drugs other than kanamycin or also in synergistic effects with another drug.

5.4 Conclusion/ Clinical Significance of the Present Study

The success of drug therapy is highly dependent on the dosage regimen. Assessment of clinical effects in the light of pharmacokinetics and plasma concentration is preferred in making decision for necessary adjustment of the dosage whenever possible [15]. The present study showing little difference in pharmacokinetics between healthy subjects and MDR-TB patients indicated that the disease itself may have less influence in altering the fate of kanamycin. Moreover, the response of patients, seen by clinical and laboratory assessment supported the important therapeutic implications for the use of kanamycin in Myanmar MDR-TB patients.

However, a great inter-individual variation in kanamycin concentration seen in the study may indicate that dosage individualization and therapeutic drug monitoring may be considered as effective approach to optimize kanamycin therapy in MDR-TB patients who did not response satisfactorily to second-line treatment. The finding of ototoxicity only in healthy volunteers may indicate that patients may well tolerate or accept the side effects of drugs better than volunteers but such information on side effects and need for compliance in tuberculosis should be given to the patients at the time of diagnosis through appropriate health education programs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgement

We owe sincere gratitude to Professor Dr. Tint Swe Latt, Rector, University of Medicine 2, Yangon, and Dr. Khin Pyone Kyi, Director General, Department of Medical Research, (Lower Myanmar) for allowing doing this study. We are also indebted to Dr. Myo Zaw, Medical Superintendent, Union Tuberculosis Institute (UTI), Aung San Township, Yangon for his kind help. The authors thank to all the staff at Union Tuberculosis Institute and all the staff at Bacteriology Research Division, Department of Medical Research (Lower Myanmar) for their kind co-operation.

References

WHO. Global tuberculosis control: a short update to the 2009 report. Geneva: World Health Organization, 2009. http://www.who.int/tb/publications/global-report/2009/update/tbu_9.pdf (accessed Feb 1, 2010).

Dye C, Williams BG. The population dynamics and control of tuberculosis. Science 2010; 328: 856–61.

WHO. Global tuberculosis control. Geneva: World Health Organization, 2010. http://whqlibdoc.who.int/publications/2010/ 9789241564069-eng.pdf (accessed Dec 15, 2010).WHO (2008). Global tuberculosis control: surveillance, planning, finances WHO report, Geneva,WHO/HTM/TB/2008.393.

Clarke JT, Libke RB, Regamey C (1969). Comparative Pharmacokinetics of amikacin and kanamycin. *Clin Pharmacol Ther*. **15:** 610-616.

Maurya V, Vijayan VK, Shah A (2002). Smoking and tuberculosis: an association overlooked. *Int J Tuber Lung Dis*, **6(11):** 941-951.

Alcaide J, Alert MN, Plans P, Parron I et al. (1996). Cigarette smoking as a risk factor for tuberculosis in young adults: a case-control study. *Tuber Lung Dis*, **77(6):** 570.

Jager DP, Altena VR (2002). Hearing loss and nephrotoxicity in long-term aminoglyco-side treatment in patients with tuberculosis. *Int J Tuber Lung Dis*, **6:** 622-627.

Peloquin CA. (2004). Aminoglycoside Toxicity: Daily versus Thrice-weekly Dosing for Treatment of Mycobacterium Diseases. *Clin Infect Dis*, **38 (1):** 1538-1544.

Phillips CW (1974). Serum gentamicin assay: a comparison of assessment of different methods. *Clin Pharmacokinet*, **27:** 447-451

Yusuke T, Reiko S, Kunihiko M (2006). Population Pharmacokinetics of Arbekacin in patients infected with Methicillin-Resistant *Staphylococcus aureus*. *Antimicrobial Agents Chemother*, **50(11):** 3754-3762

Daniel O and Osman E. Prevalence and risk factors associated with drug resistant TB in South West, Nigeria (2011). *Asian Pacific Journal of Tropical Medicine* , **Vol 4(2):** 148-151

Kanyok TP, Aaron DK (1997). Pharmacokinetics of Intramuscularly Administered Aminosidine in Healthy Subjects. *Antimicrobial Agents Chemother*, **41(5):**982-986

Shargel L, Yu ABC (1993). Introduction to Pharmacokinetics. Applied Biopharmaceutics and Pharmacokinetics, 3rd edition, Appleton and Lange, Norwalk, Connecticut, p.33-45

Blower SM, Chou T (2004). Modeling the emergence of the hot zones: tuberculosis and the amplification dynamic of drug resistance. *Nature Medicine*, **10(10):** 1111-1116

Gandhi NR, Nunn P and Dheda K(2010) Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 2010; 375: 1830–43.

WHO. Multidrug-resistant tuberculosis (MDR-TB) 2013 Update: A short update to WHO March 2013. Geneva: World Health Organization, 2013. http://www.who.int/tb/challenges/mdr/MDR-TB-FactSheet.pdf