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Full Length Article

Pharmacokinetic Studies of Rifampicin in Healthy Volunteers and Tuberculosis Patients

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ABSTRACT

This article describes the population pharmacokinetics of rifampicin in Pakistani pulmonary tuberculosis patients and healthy volunteers, to determine the variability in the pharmacokinetics of rifampicin (RMP). Subjects ingested single doses of RMP, 450 mg, under fasting conditions. Thirteen healthy and thirteen patient volunteers were selected for the studies. The typical population estimate of oral clearance was 8.7 and 11.9 L/h in patient and healthy volunteers, while the volume of distribution was estimated to be 39 and 54.14 L in tuberculosis patients and healthy volunteers, respectively. The changes in C_{max} and AUC are non-significantly different in both healthy volunteers and tuberculosis patients, while significant variability was observed for the T_{max}, which is higher in healthy volunteers compared to the tuberculosis patients. © 2010 Friends Science Publishers

Key Words: Tuberculosis; Pharmacokinetics; Rifampicin; Patients

INTRODUCTION

Pakistan is a developing country with a rapidly growing population. Over the last few years tuberculosis has become a major problem for the health system of this region of high prevalence (Anonymous, 1999; WHO, 1999). Pakistan lines number one in the Eastern Mediterranean region and eight in the top tuberculosis prevalence countries. The incidence of tuberculosis was 254 cases per 100 000 population in 1995 and has been estimated to 181 cases /100,000 population in 2008 (WHO, 2008). Seventy five percent of the cases are in the age group 15–59 years, the most economically productive sector of society. Thirty three percent of all cases are extra-pulmonary (Hussain & Khan, 1998), indicating 67% cases as pulmonary tuberculosis. TB is perceived as a dangerous, infectious and incurable disease in Pakistan (Liefooghe, 1995). Pulmonary tuberculosis is a very common cause of mortality in our country (Iqbal & Mohammad, 2000). Keeping in mind its high prevalence all aspects for treatment of the disease need to be thoroughly studied. The standard short-course treatment of tuberculosis comprised of isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), plus either ethambutol (EMB) or streptomycin until vulnerability data are obtainable (American Thoracic Society, 1994).

Rifampicin binds to RNA polymerase and interfere the synthesis of mRNA (Telenti, 1998). Resistance develops in Mycobacrteria if a specific region for the RNA polymerase subunit is mutated. The gene rpoB if mutated is responsible for most of the resistance in mycobacteria (Miller *et al*., 1994). Rifampicin is the key sterilizing' component of extremely efficient short course antituberculosis regimen and it is likely that rifampicin's sole role is its aptitude to kill semi-dormant Tubercle bacilli when they experience sporadic bursts of metabolism and growth (Wilkins *et al.,* 2008). It also avoids the appearance of resistance to other fixed dose combination drugs (Mitchison, 1992).

To guarantee optimal use and to facilitate scientific bioequivalence assessment of rifampicin, it is necessary to evaluate its pharmacokinetics under local environmental circumstances, using precise and responsive modern analytical techniques and better study designs. The drugs used in Pakistan are imported from foreign in raw or finished form because of insufficient indigenous production. All studies on these drugs are conducted on animals and humans, which are different from those of local. The studies conducted over several years under indigenous settings have exposed differences between the overseas and local species of animals. In recent years the study of drug disposition in population variability has got increasing consideration. Genetic diversity in pharmacokinetic actions has been well recognized (Castaneda *et al*., 1993; Vesell, 1997). Racial disparity environmental aspects and nutritional routines have been depicted to manipulate actions of several drug metabolizing enzymes (Min *et al*., 2000; Kumar *et al.,* 2004).

The studies were conducted at the District Head Quarters Hospital Faisalabad, Pakistan. The participants

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comprise of thirteen pulmonary TB patients and thirteen healthy volunteers. All participants were receiving standard anti-TB regimens. The study was approved by the institutional ethics committee and informed written consent was obtained from all the participants. We examined the pharmacokinetics of RMP in healthy volunteers and tuberculosis patients under fasting conditions (two replicates). This study describes the concentrations in serum and the pharmacokinetic behaviour under optimal conditions and the results can be used as benchmarks for comparison with those for samples obtained in other clinical settings.

MATERIALS AND METHODS

A prospective pharmacokinetic study was conducted among thirteen healthy and thirteen patients with pulmonary tuberculosis at the District Head Quarters (DHQ) Hospital Faisalabad, Pakistan. The study protocol was approved by the chest specialist (Dr. Muhammad Sadiq) from the same Hospital. Written consent taken from each volunteer before inclusion into pharmacokinetic studies. The drug products (FDC containing isoniazid, rifampicin, pyrazinamide & ethambutol) employed in the studies were those routinely prescribed by the medical officers in the hospital (Rifa-4® Schazoo Pakistan). The antituberculosis drug rifampicin was administered under fasting conditions. After drug ingestion blood samples were obtained immediately before and at 0.5, 1, 2, 3, 4, 6, 12 and 24 h after drug ingestion. Prior to centrifugation of samples at 4000 rpm using centrifuge machine (YJ03-043-4000 China) samples were temporarily stored in an ice box. Centrifugation of all samples to get plasma was completed within thirty minutes of collection and stored in 2 mL polypropylene tubes to store in -80ºC freezer till further analysis by HPLC (Waters, 600 E). Protocol proposed by Kumar *et al.* (2004) was adopted to determine the plasma concentrations of rifampicin by HPLC with the aid of tuneable UV detector (Waters 484) (Kumar *et al.*, 2004).

Analysis of rifampicin: From each sample and standard, 500 µL of plasma was taken in the microfuge tube and 500 µL acetonitrile was added and centrifuged at 10,000 rpm for 10 min. It was filtered through 0.22 µm membrane (13 mm) and injected 25 µL to column. A blank was prepared by taking 500 µL of drug free urine in the same manner. Mobile phase was prepared by using 0.02 M phosphate buffer pH 3.6 and acetonitrile (55:45 v/v). Buffer was filtered through Whatman filter paper and added 450 mL acetonitrile in 550 mL buffer. Mobile phase was filtered (cellulose acetate filter diameter 47 mm, pore size 0.45 µm, Sartorius AG. 370700) and sonicated (EYELA-Sonicator) for 12 min.

Standard curve for rifampicin: The reference drug to construct the regression line was obtained from Sigma. Concentration of rifampicin was calculated in plasma by using the linear regression equations Y = 14.984X-8.028.

Pharmacokinetic profiles: Two compartmental analyses was used to compute the peak drug concentration C_{max}, the time to C_{max} (t_{max}), the plasma half-life ($t_{1/2}$), the area under the curve until the last measurable concentration (AUC0–24), and the area under the curve extrapolated to infinity (AUC0–∞). Concentrations in plasma below quantification lower limit were treated as zeros in averaging the concentration at a given collection time. The area under the plasma concentration versus time curve (AUC) from time zero to the time of the last quantifiable concentration ($AUC_{0\text{-}t^*}$) was determined by linear trapezoidal rule. The last quantifiable concentration was designated C*. The AUC from time zero to infinity ($AUC_{0\text{-}\infty}$) was determined as $AUC_{0\text{-}t^*} + C^*/\beta$ with β. APO software MW/PHARM version 3.02 (Holland, 1987) was used to construct candidate pharmacokinetic model. The model included the zero order absorption time, T_{abs} (mg/h), the absorption lag time (T_{lag} [hours]), the volume in the central compartment, V_1, (L/kg), intercomparmental transfer rate constant, K_{21} (l/h) and the α and β elimination rate constants, (l/h). The rate constant K_{10} was calculated as $(\alpha \times \beta)/K_{21}$, K12 was calculated as $[(\alpha + \beta)-K_{21}-K_{10}]$ and total body clearance CL, (litters per hour) was calculated as (V_1 bx K_{10}). The steady state volume of distribution $V_{ss,}$ (litters per kilogram) was calculated as $V_1 \times (1+K_{21}/K_{12}$. The terminal elimination half life $t_{1/2}$ was calculated as $\ln(2)/\beta$. (MW/PHARM version 3.02).

Biochemical and demographic data: The values of demographic data (age, body weight, height, blood pressure & body temperature) for the thirteen healthy and patient volunteers are presented in the Fig. 1 and 2, respectively. The biochemical parameters (albumin, globulin & creatinine) were also determined.

Statistical analysis: The mean values and standard deviation (SD) for each parameter were calculated and the results have been presented in tables and graphs Using Microsoft excel version 2002. Comparisons of the patient and healthy volunteer parameters were carried out with the help of t-test (Steel *et al.*, 2006).

RESULTS AND DISCUSSION

The plasma concentration versus time data has been presented in Fig. 3 for both healthy volunteers and TB patients after oral administration of 450 mg of RMP in the form of FDC tablets. Concentration of drug increased up to 2 h in volunteer patients, while it declined gradually in healthy volunteers after 4 h. The concentration of rifampicin remain above the level of MIC (0.25 µg/mL (Weis *et al.*, 1994) in healthy volunteers and TB patients twelve hours post dose (Fig. 3). The rifampicin bioavailability is affected by a number of factors as the manufacturing process (Cavenaghi, 1989), food (Siegler *et al.*, 1974) and excipients (Boman *et al.*, 1975). In our studies a sharp downfall ($P<0.05$) in the concentration of drug was observed in the patient volunteers compared to healthy ones as a result the

Table I: Pharmacokinetic parameters of RMP in TB patients and healthy volunteers after oral administration of FDC anti TB drugs

Kinetic parameters	Patient volunteers (n = 13)	Healthy volunteers (n = 13)
AUC (h.mg/mL)	44.4 ± 13.6	38.882NS ± 6.988
AUC poly (t=24)	44.2 ± 13.6	38.388NS ± 7.048
AUC trap (t=24)	48.9 ± 15.1	44.979NS ± 7.136
CL (L/h)	8.7 ± 2.7	11.965* ± 2.198
Vd comp.1 (L)	25.5 ± 11.5	37.807NS ± 16.309
Vd st.state (L)	33.0 ± 14.0	44.970NS ± 16.266
Vd (L)	39.0 ± 14.7	54.149NS ± 16.646
half life ph.1 (h)	0.7 ± 0.2	0.739NS ± 0.416
half life ph.2 (h)	2.8 ± 1.0	3.114NS ± 0.783
Rate const. K_{10} (/h)	0.4 ± 0.2	0.360NS ± 0.116
Rate const. K_{12} (/h)	0.2 ± 0.1	0.097NS ± 0.067
Rate const. K_{21} (/h)	0.5 ± 0.2	6.735NS ± 13.337
MRT [h]	5.0 ± 1.5	5.596NS ± 0.504
Ka [/h]	0.7 ± 0.3	0.635NS ± 0.265
Absorption half life [h]	1.1 ± 0.7	1.339NS ± 0.642
t_{max} [h]	1.7 ± 0.5	2.105* ± 0.298
C_{max} [µg/mL]	6.6 ± 2.0	5.518NS ± 0.785

NS= Non-significant; * = Significant; Mean±SD
(AUC= Area under curve, CL= Clearance, Vd comp 1= Volume of distribution of compartment 1, Vd st state = Steady state volume of distributuion, MRT= Mean residential time, t_{max} = Time to reach maximum concentration, C_{max} = Maximum concentration)

Fig. 1: Mean ±SD values for demographic data of healthy volunteers

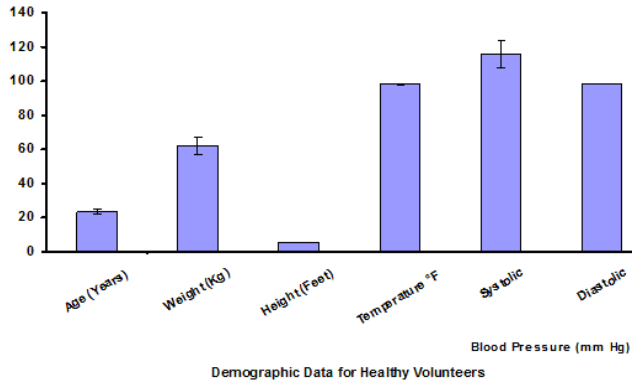
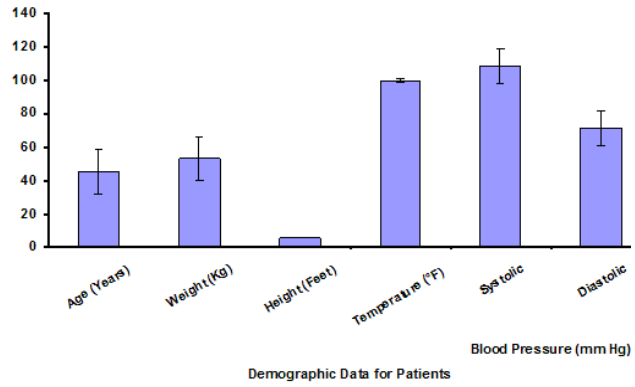


Fig. 2: Mean ±SD values for demographic data of patient volunteers



area under the concentration curve was lowered significantly (P<0.05) in tuberculosis disease compared to healthy volunteers, indicating the involvement of the disease condition in the bioavailability of the drug (Fig. 3). All the three interrelated parameters like half life, body clearance and volume of distribution between two populations did not show significant (P>0.05) difference (Table I). The time to reach maximum concentration was same in both populations; however the concentration of drug at that time was significantly higher in healthy volunteers (Table I). Oral doses of rifampicin taken on an empty stomach are well absorbed as described earlier (Nitti *et al.*, 1977).

In another study carried out in children, it has been indicated that only about 50% of oral doses are absorbed (Koup *et al.*, 1986). After oral administration, maximum mean plasma concentrations of 7.1 and 5.1 µg/mL were achieved at approximately in 1.8 h for TB patients and healthy volunteers, respectively in the present studies (Fig. 3). In contrast to our results C_{max} values for three different preparations of Rifampicin by Pahkla *et al.* (1999) ranged from 10.32-11.58 µg/mL and by Peloquin *et al.* (1999) the C_{max} value was 10.54 µg/mL. The t_{max} values in these studies are in line with the work of McIlleron *et al.* (1999) presenting t_{max} values of 1.6-2.3 while Peloquin *et al.* (1999) reported a t_{max} value of 2.4. Chouchane *et al.* (1995) reported that peak rifampicin serum concentrations were achieved within about 2 h when the drug was taken on an empty stomach, but the absorption takes more time and it is incomplete if it is ingested with food (Buniva *et al.*, 1983). Absorption and elimination pharmacokinetics of rifampicin are not affected, if the drug is taken in association with isoniazid and ethambutol (Acocella *et al.*, 1988).

Almost 85% of serum rifampicin concentrations were protein bound with bound drug as 86.1±l.5%, which was significantly lower than healthy subjects (88.9±0.9) (p<0.001). The plasma albumin concentrations were lower in patients than the healthy subjects (44.0 *vs.* 47.5 g/L p<0.05), but were still in the accepted normal range (Boman & Ringberger, 1987). Similar results were observed for the albumin concentrations in the healthy volunteers and TB patients in the present study, where patients had significantly lowered concentrations compared to healthy volunteers (34 *vs.* 59 g/L p<0.05) (Fig. 4). The level of unbound rifampicin in the plasma is likely to be increased by simultaneous administration of other antitubercular drugs, which could lead to prolonged drug action consequently (Polasa & Krishnaswamy, 1987). A positive correlation between Protein binding of rifampicin and $AUC_{0-\infty}$ (r=0.5, p<0.05) showed that protein binding influence the bioavailability (AUC) of drugs. As rifampicin binds only to the albumin (Polasa & Krishnaswamy, 1984), a decrease in the level of albumin for the patients was observed in the present studies. The area under the concentration curves (AUC0-8) after giving standard single or repeated daily doses of 600 mg (or about 10 mg/kg)

Fig. 3: Plasma concentration versus time data of Rifampicin in healthy volunteers and TB patients

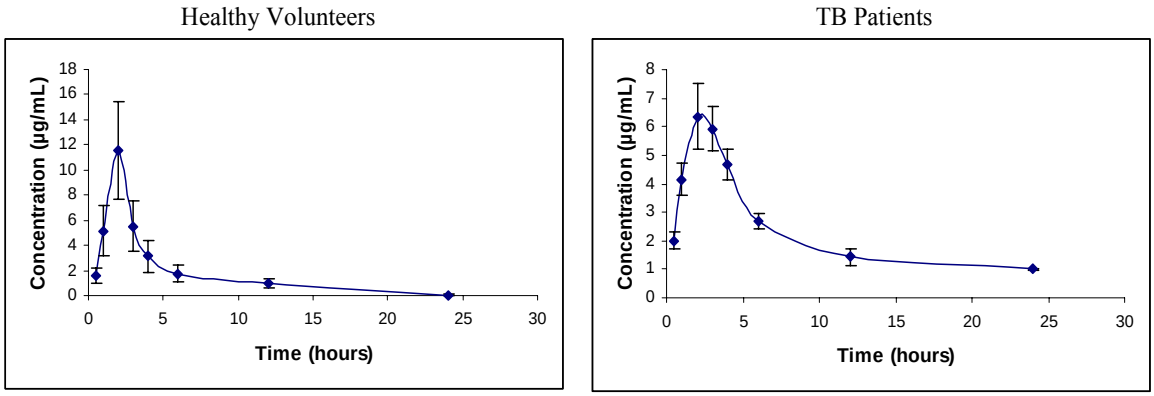
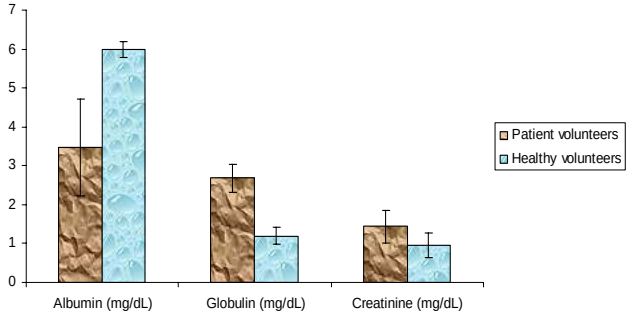


Fig. 4: Comparison of albumin, globulin and creatinine levels in healthy and TB patient volunteers



rifampicin ranges from 34.8 to 67.3 mg. h/mL (Gurumurthy *et al.*, 1990; Gurumurthy *et al.,* 1992; Zwolska *et al.,* 1998). The results of rifampicin bioavailability in our study coincided with that reported in the above mentioned studies (AUC_{0-24}) for both diseased (44 mg. h/mL) and healthy volunteers (38 mg. h/mL), but much lesser compared to previous studies e.g., AUC 0-8 =77.9, 82.7 and 68.44 mg. h/mL (Pahkla *et al.*, 1999), AUC 54 (Peloquin *et al.*, 1999) and AUC = 52-74 mg. h/mL (McIlleron *et al.*, 1999) are severe as they can lead to considerable treatment failure for short-course regimen and support the selection of both isoniazid- and rifampicin-resistant *M. tuberculosis* (Fox, 1990). Jayaram *et al.* (2003) reported that the antimicrobial activity of rifampicin is related to the ratio of the AUC to the MIC. The AUC: MIC ratio in patients and healthy volunteers in our studies is 177.7 and 155.5.

According to Heifets, a rough measure of the potency of a drug is the ratio of the maximal concentration and the MIC (C_{max}: MIC ratio). A ratio 4 indicates probable effectiveness (Heifets, 1991). This ratio is also important to prevent emergence of resistance (Gumbo *et al.*, 2007). In our studies the Cmax/MIC ratio in healthy volunteers is 22.07±3.2 and for TB patients it is 26.3±7.8 after oral administration of 450 mg rifampicin. Chan *et al.* (1992) and Sirgel *et al.* (1993) reported that the antituberculosis activity of the RMP is dose dependent.

CONCLUSION

The changes in C_{max} and AUC are non-significantly different in both healthy volunteers and tuberculosis patients, while significant variability was observed for the T_{max}, which is higher in healthy volunteers compared to the tuberculosis patients. A positive correlation between protein binding of rifampicin and $AUC_{0-\infty}$ ($r=0.5$, $p<0.05$) showed that protein binding influence the bioavailability (AUC) of drugs.

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