Assessment of Bioavailability of Rifampicin as a Component of Anti-tubercular Fixed Dose Combination Drugs Marketed in Pakistan

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Abstract

The World Health Organization (WHO) recommended DOTS program recommends treatment of TB with a combination of three to five drugs. However, international bodies like the WHO and IUTLD (international union against tuberculosis and ling disease) recommend the use of only those fixed dose combinations that have proven in vivo bioavailability. The present study was conducted to test the in vivo bioavailability of some of the formulations currently marketed in Pakistan on twenty six healthy volunteers as a three sequence, three period cross-over study. Rifampicin was administered in three different formulations out of which one (Formulation A) acted as a standard against which the other two formulations; Formulation B (Fixed dose combination without pyrazinamide) and formulation C (Fixed dose combination with pyrazinamide) were tested. Thirteen blood samples including a pre-dose sample were drawn over a period of 24 hours. Plasma samples were analyzed for rifampicin concentration by an HPLC method and critical pharmacokinetic parameters were calculated. Although, based on the confidence intervals for the ratios of geometric means of pharmacokinetic parameters none of the test formulations B or C could be declared bioequivalent, nevertheless effective formulations for the treatment of TB in Pakistan.

Introduction

A current assessment has reveled that tuberculosis (TB) continues to be a leading killer disease amongst adults and children worldwide and remains to be a socio-economic problem that impedes human development. In the year 2004, World Health Organization (WHO) estimated that there exist around 14.6 million cases of TB worldwide and 1.7 million deaths due to this disease annually, 8.9 million new cases of TB were registered in that year alone. The developing countries and populations especially with HIV infection suffer disproportionately as 250,000 deaths were reported due to TB/HIV co-infection. In addition, rise of resistant tuberculosis strains and MDR-TB were reported in 102 of 109 settings surveyed (World Health Organization, 2006). It has been estimated that between 2002 and 2010, approximately one billion people will be newly infected, over 150 million people will get sick and 36 million will die of TB if control is not further strengthened (World Health Organization, 2006). Current morbidity data show that TB is the most prevalent infectious cause of death, being responsible for one in seven adult deaths and one in four preventable adult deaths worldwide (Gandy and Zumla, 2002). In Pakistan, like other developing countries, the case is no less alarming. Pakistan faces the grim facts that it has the sixth highest Tuberculosis burden globally and also accounts for 43% of TB cases in the Eastern Mediterranean region comprising 23 countries. In Pakistan- a poor and crowded country of 160 million people, some 1.5 million suffer from TB. WHO estimates incidence of sputum positive tuberculosis in Pakistan is 80/100,000 and 177/100,000 for all form of tuberculosis. More than 250,000 persons acquire active TB disease every year (Bio-Statistic Division/PHC cell, 2005). In 2001 a national emergency on TB was declared by the Federal Ministry of Health, Islamabad. Treatment of Tuberculosis currently involves administration of a combination of rifampicin (R, RMP), Isoniazid (H, INH), pyrazinamide (Z, PZA) and Ethambutol (E, EMB) for the initial 2 months, followed by RMP and INH for 4 months. The World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUTLD) recommended the use of fixed dose combinations (FDCs) of anti-tuberculosis drugs to prevent monotherapy and drug resistance (World Health Organization, 1999). The FDCs provide many advantages over single drug products but there have been numerous reports that a fixed dose combination of anti- TB drugs very often leads to poor bioavailability of RMP component although there also have been reports about these products performing satisfactorily (Shishoo et al., 2001; McIlleron et al., 2002). In contrast to other medications, anti-TB drugs are stable under storage conditions, so sub-standard levels are usually not caused by instability. Patients receiving a lower therapeutic dose due to a substandard anti-TB drug could lead to drug resistance and treatment failure despite 100% adherence to the treatment regimen (Singh et al., 2000; Ashokraj et al., 2005; Ashokraj et al., 2004; Agrawal and Panchagnula, 2004; Laserson et al., 2001). The World Health Organization (WHO) and International Union against Tuberculosis and Lung Diseases (IUATLD) issued a joint statement in 1994 pointing out that anti-TB FDC products should only be used if the bioavailability of at least the rifampicin component has been demonstrated (IUATLD/WHO, 1994).

Methods

Study design, subjects and data analysis

This study was approved by the ethics committee of Quaid-e-Azam University, Islamabad, Pakistan. It was randomized crossover study and spanned over a total of three periods with one week washout time between two subsequent periods. After careful screening and interview, twenty four healthy male volunteers aged between 20-30 years were recruited as study subjects by ensuring selection criteria described in the Evaluation of Medicines for Human Use by the European Agency for the Evaluation of Medicinal Products (The European Agency for the Evaluation of Medicinal Products, 2001). Blood examinations for hemoglobin and liver function i.e. bilurubin, creatinine and alkaline phosphatase were carried out before and no change was observed. Each volunteer was allotted a study number completely at random and asked to proceed to the study site with an empty stomach (an overnight 8 hour fast). The formulations were administered to the subjects in therapeutic doses with a glass of water (app. 150 ml.). The subjects were denied any food or drink except for the light meals served two and six hours after the dosing respectively. All the drugs used for the bioavailability studies were procured from the market, reference standards for all the four drugs in the study were provided by Wyeth-Lederle Pakistan, Ltd. All the chemicals used in the study were manufactured by Sigma Aldrich, Germany. Distilled water was used wherever required in the study. Values for the plasma concentration- time curve for Rifampicin were plotted on a semi-log curve using Graphpad Prism® V 5.01. WinNonlin (Pharsight, USA) was used to calculate the pharmacokinetic parameters. Bioequivalence testing was then performed in WinNonMix. An ANOVA was carried out for the natural logarithms each of the three parameters in order to test for sequence, period, treatment or subject effects, and bioequivalence was determined by calculating the ratio of the geometric means of the parameters and confidence intervals for the ratios.

Instrumentation

Shimadzu LC-9A HPLC system was used in the study. A C-18 (3.9x30mm) reverse phase HPLC column manufactured by Waters Associates was used for the study. Dissolution studies were carried out on ERWEKA DT6 dissolution apparatus. Other instruments included were ERWEKA ZT3-A disintegration apparatus, Abbott Diagnostics Centrifuge machine Cat. No. 9527-16, Torika Vortex Mixer model MA-1, EYELA Vacuum drying system with Vacuum Oven (VOS-300) and refrigeration system (UNI-TRAP UT-50L), Shimadzu (Libror-AEU-210) electronic balance, TOA pH meter (HM 50-S) manufactured by TOA electronics, Japan and Shimadzu (UV-2100S) Spectrophotometer.

Mobile phase

Methanol-sodium phosphate buffer (final pH 5.4) used in a v/v ratio of 65:35 at a flow rate of 1ml/min. was used as the mobile phase (Panchagnula et al., 1999). The mobile phase was sonicated for 10 minutes before it was used. This combination of methanol-buffer resulted in well resolved peaks for rifampicin.

Study formulations

The details of the study formulation i.e. Formulation A which is the reference and includes separate formulations, formulation B which is a fixed dose combination from a reputed local manufacturer and formulation C again is a fixed dose combination from a multinational drug manufacturer are given in Table 1, Table 2 and Table 3.

Blood sample collection and analysis

A canula was inserted in the cubital vein of each of the volunteers and periodic sampling was done with the help of a syringe. 5ml blood was drawn during each sampling and transferred to heparinized tubes. Sampling included a predose sample and subsequent samples drawn at 15 min, 30 min, 1hr, 1.5 hr, 2 hr, 2.5 hr, 3 hr, 4 hr, 6 hr, 8 hr, 10 hr, and 12 hr. intervals. After collection the blood was immediately centrifuged at 4000 rpm for 15 minutes and the plasma was collected in eppendorf tubes and frozen at -20℃ until analyzed. High Performance liquid Chromatography (HPLC) was used for the analysis of rifampicin in the plasma samples.

Standard curve

Calibration stock solution of rifampicin in a concentration of 1mg/ml was prepared in methanol. The stock solution was then diluted to

Drug	Strength	Dosing	Trade Name	Manufacturer	B. No.	MFG.	Exp.
Rifampicin	600 mg.	1 Tab.	Lederiff	Wyeth-Lederle	03C0177	03-2004	03-2008
INH	100 mg.	3 Tabs.	Generic	Unexo Labs.	09646	07-2004	07-2008
Pyrazinamide	500 mg.	3.2 Tabs.	PZA-CIBA	Novartis	567	03-2003	02-2008
Ethambutol	400 mg.	3 Tabs.	Myambutol	Wyeth-Lederle	02N0731	12-2004	12-2008

Table 1: Formulation A (Reference; Separate Formulations).

Drug	Strength	Dosing	Trade Name	Manufacturer	B. No.	MFG.	Exp.
Rifampicin	150 mg.						
INH	75 mg.	4 Tabs.	Rifatol	Schazoo Laboratories.	RIT 152	05-2004	05-2008
Ethambutol	300 mg.						

Table 2: Formulation B (Fixed Dose Combination).

Drug	Strength	Dosing	Trade Name	Manufacturer	B. No.	MFG.	Exp.
Rifampicin	150 mg.						
INH	75 mg.	4 Tabs.	Myrin-P Forte	Wyeth-Lederle	03G0195	07-2005	07-2008
Pyrazinamide	400 mg.						
Ethambutol	275 mg.						

Table 3: Formulation C (Fixed Dose Combination).

Formulation	Assay Results	%age Dissolved in		
		15 min.	30 min.	45 min.
Lederiff (A)	108%	76.5%	97.6%	108%
Rifatol (B)	109.9%	14.4%	54.4%	101.5%
Myrin P Forte (C)	108.9%	30.6%	61.3%	93.6%

Table 4: Assay and Dissolution Studies.

100 μg/ml with methanol to make a working stock solution. From this working stock solution, calibration standards were made in plasma in concentrations of 1,2,3,4,5,6,7,9,10,12 and 15 μg/ml. The standard curve obtained with these calibration standards showed a correlation coefficient of 0.998 (Figure 1).

Extraction of rifampicin from plasma samples

Stored frozen plasma was thawed on ice and 200 μg volumes were taken in a 1.5 ml eppendorf tube. 1ml methanol was added to the tube and the tube was vortexed for 2 minutes. The sample was then centrifuged at 10000 rpm for 10 minutes and 800 ml of the clear supernatant was separated in another tube. The sample was dried in a vacuum oven and the residue left after drying was reconstituted with 200 μg mobile phase. 100 μl was injected into the HPLC system for analysis. Analyte detection was done with a UV detector set at 254nm.
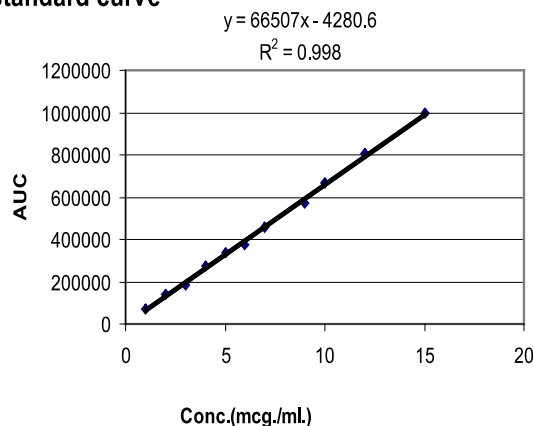

Figure 1: Standard Curve of rifampicin standard.

	Cmax	AUC$_{all}$	AUC$_{0-\infty}$
Period	0.1802	0.2527	0.3480
Sequence	0.3449	0.1290	0.1135
Treatment	0.0507	0.2904	0.9056
Subject	0.2736	0.1611	0.2391

Table 5: Results of ANOVA (p-values) with Formulation A as reference product and Formulation B as test product.

|	Ratio (%)	90% Confidence Interval
Cmax	133.7658	(105.5780, 169.4794)
AUC$_{all}$	114.3210	(91.8858, 142.2341)
AUC$_{0-\infty}$	101.7369	(78.5368, 131.7905)

Table 6: Results of bioequivalence testing with Formulation A as reference product and Formulation B as test product.

	Cmax	AUC$_{all}$	AUC$_{0-\infty}$
Period	0.4357	0.6315	0.6832
Sequence	0.0460	0.1694	0.2433
Treatment	0.0325	0.1621	0.2650
Subject	0.1266	0.1367	0.2313

Table 7: Results of ANOVA (p-values) with Formulation A as reference product and Formulation C as test product.

	Ratio (%)	90% Confidence Interval
Cmax	120.9730	(105.3543, 138.9071)
AUC$_{all}$	114.7395	(97.2339, 135.3968)
AUC$_{0-\infty}$	115.6588	(92.4096, 144.7573)

Table 8: Results of bioequivalence testing with Formulation A as reference product and Formulation C as test product.
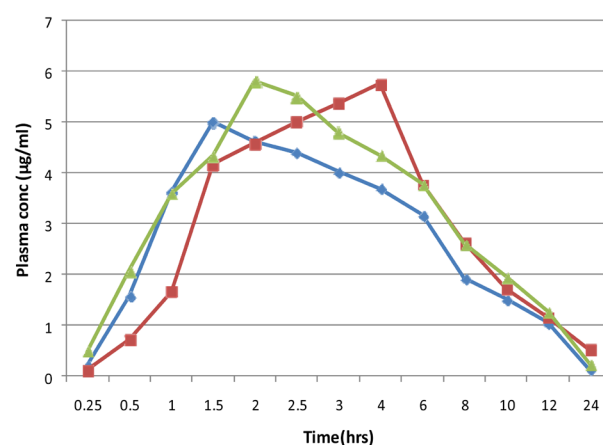
Time	Conc.1	Conc.2	Conc.3
0.25	0.226044	0.120538	0.502419
0.5	1.572894	0.717064	2.071375
1	3.639846	1.670332	3.597683
1.5	5.007513	4.171079	4.338442
2	4.626576	4.584823	5.81492
2.5	4.400809	5.008724	5.515726
3	4.027521	5.3751	4.798195
4	3.690477	5.756782	4.35989
6	3.174343	3.777431	3.781082
8	1.928214	2.629347	2.60821
10	1.516612	1.718295	1.951792
12	1.044628	1.164749	1.264701
24	0.13732	0.5208	0.2491

Plasma Conc(μg/ml) [A]
Plasma Conc(μg/ml) [B]
Plasma Conc(μg/ml) [C]

Figure 2: In-vivo Concentration- Time Profile.

In-vitro dissolution studies

The dissolution profile of the study formulations was performed on USP apparatus 1 working at 50 rpm and gastric fluid was used as dissolution medium. Samples were drawn at 15, 30 and 45 minute intervals and were analyzed at 475nm on UV spectrophotometer (USP, 24th Edition).

Results

The study was started with twenty six healthy male volunteers from Islamabad, Pakistan. Due to personal reasons two of the volunteers could not continue with the study after the first period so the study was concluded with twenty four subjects. The chemical assay and dissolution profiles of three formulations containing rifampicin are given in Table 4. Values for the plasma concentration-time curve for all the three formulations are presented in Figure 2. The results of ANOVA (p-values) with formulation A as reference product and formulation B as test product did not reveal any significant effects, although the treatment effect on C$_{max}$ was almost statistically significant. Results of bioequivalence testing with Formulation A as reference product and Formulation B as test product showed that for each of the parameters C$_{max}$ and AUC, the mean value of Formulation B was larger than that of Formulation A, and confidence intervals for none of the parameters fell within the standard limits of 0.8-1.25 (Dotevall and Ekenved, 1976) to be declared bioequivalent (Table 5 and Table 6). Results of ANOVA (p-values) with Formulation A as reference product and Formulation C as test product shows statistically significant effects on C$_{max}$, a sequence effect and a treatment effect. Results of bioequivalence testing with Formulation A as reference product and Formulation C as test product revealed that for each of the parameters C$_{max}$ and AUC,

the mean value of Formulation C was larger than that of Formulation A, and confidence interval for none of the parameters were within the standard limits (0.8-1.25), hence the two formulations cannot be declared bioequivalent (Table 7 and Table 8).

Discussion

Although as a result of this study none of the test products can be declared bioequivalent to the standard product; there was no problem in the bioavailability of RIF with any of the two FDCs tested which is contrary to our initial assumption. Looking at the results of invitro dissolution studies for the products it can easily be appreciated that assay results are in fact very similar among the three products and the superior bioavailability of RIF from the FDCs cannot be attributed to this factor. However, this effect can be explained on the basis of dissolution time profile of the two preparations. At 30 minute time interval (which is equal to the gastric emptying time) Formulation A has already released almost its entire contents while formulation B and formulation C show 54.4% and 61.3% drug release respectively. Since theoretically the drug released in the basic environment of the duodenum has greater absorption as compared to the proportion of the drug released in the acidic environment of the stomach, more of RIF "dumped" from the standard formulation would reach blood and hence be available for elimination and since elimination rate constant has first order kinetics it would be eliminated faster than RIF from the test formulations, which have a more "sustained" kind of drug release pattern. This is depicted by a larger elimination rate constant for Formulation A than the two FDCs (data not shown). This phenomenon needs to be looked into and may provide some insight into developing dosage forms with better bioavailability profiles.

The bioavailability of rifampicin from the formulations can also be explained on the basis of physiochemical properties of the drugs and dosage forms. Since rifampicin tablets are film coated, this dosage form presents all the potential problems of compressed tablets that impose a physical barrier between the gastrointestinal fluid and tablet containing the drug. The prerequisite for the absorption of rifampicin from film coated tablets is dissolution of the coat and then dissolution of the core of the tablet. It may be one of the possibilities that the difference found in bioavailability parameters and superiority of formulations B and C to formulation A is probably due to the difference of coating material employed (Winne, 1977; Suzuki et al., 1970; Blomberg et al., 2001), in addition to compression force applied for making core of the tablet and other excepients used. The other probable reasons such as inherent variation in the absorption of rifampicin and the extent of metabolism may not be the contributory factors for the altered bioavailability of rifampicin when determined by controlled bioequivalence trials. In randomized crossover designs which was employed for this study, every volunteer acts as his own control and hence, gastric emptying time, pH of the stomach, rate of metabolism and other individual variations have a minor role.

It has been reported that increased bioavailability of rifampicin from FDC formulations when compared to rifampicin alone products. In addition, generic formulations of rifampicin (rifampicin-alone) have also shown variable bioavailability (Laing et al., 1999). In recent years, the problem of bioavailability associated with generic formulations of rifampicin was again highlighted by McIlleron et al. (2002) who found that two rifampicin capsule formulations showed reduced blood concentrations and were responsible for failure of T.B treatment. In this regard, reduced blood concentrations from the "rifampicin-only" capsules indicate that apart from the manufacturing variables, the raw material also needs to be optimized. Another probable reason for difference in bioavailability may be the polymorphism of rifampicin, however no reports could be found that state the solubility studies of these polymorphs, and hence the reason may only be speculative. It is very difficult to correlate in-vitro dissolution -time profile with the in vivo results as the rifampicin molecule is a zwitterions with two pKa values 1.7 & 7.9 (Fourie et al., 1999; Howes et al., 2007) and this shows a highly complex pH dependent solubility and thus absorption profile especially in the pH range that exists across the GI tract (pH 1.2-7.4). Since the peak plasma levels achieved by formulation B and C are well within the normal Cmax levels specified in the literature 6 ± 3.5 μg/ml (Richard et al., 2006) well above the effective MIC concentration of 0.005-0.2 μg /ml after a usual 600 mg dose, hence the drug (rifampicin) shows no bioavailability problems from the fixed dose combinations. Thus it can safely be concluded that both the fixed dose formulations analyzed in this study either from local company or multinational have no rifampicin bioavailability problems and the use of such formulations with proven bioavailability will help in effective control and management of tuberculosis in Pakistan.

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