COMPARATIVE BIOAVAILABILITY STUDIES OF RIFAMPICIN

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Introduction

The present study was undertaken to compare in a cross-over design in both males and females, the bioavailability of rifampicin, when given in a combined formulation with isoniazid, between an established standard product and a locally produced generic product.

Methodology

The study was performed on 12 healthy male volunteers with a mean age of 23 yrs (range 22-24 yrs) and a mean body weight of 75 Kgs (range 60-90 Kgs) and on12 healthy female volunteers with a mean age of 22 yrs (range 20-26 yrs) and a mean body weight of 54 Kgs (range 45-63 Kgs). Each volunteer had no contraindication to rifampicin administration and none of the subjects was on any other medication for the last 15 days prior to the study or during the study. A cross-over design was adopted with a washout period of one week between the two treatments. The study was performed after a single dose of 600mg rifampicin 300mg isoniazid combination (adult dose) of the two formulations [Rimactazid®(batch no. 02330)- Ciba-Geigy ; Rifampicin & Isoniazid Tablets (batch no. 0011) - Pharmamed]. The single oral dose of the combined formulation was administered to each subject with 200mls of water on an empty stomach. The study was carried out using two different means of sample collection:

A. Saliva Sampling B. Urine Sampling

A.Saliva Sampling

The observations that salivary drug concentration is often proportional to plasma drug concentration led to the use of saliva as a non invasive technique for obtaining pharmacokinetic data.

Measurement of drug concentration in saliva offers several advantages over similar measurements performed in plasma. In the first place, many specimens can be obtained noninvasively, without loss of blood or exposure to discomfort and potential skin irritation and hazards of infection as with repeated venepunctures. Secondly it does not require attendance of medical staff, but more importantly, is the fact that the drug concentration in saliva represents the free fraction of drug, whereas data on concentration in plasma represents both the free and protein bound forms of the drug. Only free forms of the drug can produce a pharmacological response, so measurements of drug concentration in saliva are more therapeutically meaningful (Dvorchik *et al*, 1976; Paxton, 1979)

B.Urine Sampling

Urine sampling was used to monitor the cumulative amount of drug which was excreted in the urine. This aspect was then analyzed using cumulative urinary excretion data and applying this pharmacokinetic principle in estimating the difference, if any, between the two products in both males and females.

Analysis of Salivary Drug Concentrations

Saliva samples were collected in sterile containers at 0, 1, 2, 4, 6, 8, 12 and 24 hours after drug administration. All samples were processed immediately after collection. Rifampicin concentrations were determined by the plate diffusion assay employing a strain of *Staphylococcus aureus* (subgroup1, NCTC 10702) resistant to streptomycin and antibiotics (including isoniazid) other than rifampicin (Gurumurthy *et al*, 1990). Rifampicin standards ranging from 0.15 to 600 μg/ml were set up in quadruplicate and the concentrations of the drug in saliva samples were obtained from the regression line of the diameter of the zone of inhibition on log concentration of the standard.

Calculation of Pharmacokinetic Variables

The peak concentration (C_{max})was the geometric mean of the highest concentration in individual volunteers. The time to reach the peak concentration (T_{max}) was the mean of the values obtained for each individual. Exposure was calculated as the area under the concentration-time curve (AUC).

Analysis of Urinary Drug Concentrations

Since relatively large amounts of unchanged rifampicin are excreted in urine and since it has been shown that the AUC under the plasma concentration plot is directly proportional to the amount excreted in urine during the same time interval, the bioavailability of rifampicin from oral dosage forms may be assessed more conveniently by measuring the urinary elimination spectrophotometrically (Brechbühler *et al*, 1978).

Urine was collected at 0, 1. 2, 4, 6, 8, 12, 24, 28, 30 and 32 hours after administration of the adult dose as discussed earlier. The volunteers emptied their bladders immediately before swallowing the doses (at time 0) and all urine samples were collected and their volumes were also noted. The rifampicin concentration and consequently the amount of drug excreted was determined by the method adopted by Brechbühler *et al*, (1978).

In brief this method involved the extraction of rifampicin from the urine into toluene and then measuring the optical density of the extract spectophotometrically at 478nm and correlating the rifampicin concentration by means of calibration curves. The toluene extract of urine from volunteers contained not only unchanged rifampicin but also the metabolite 25-desacetylrifampicin, and since this shows the same absorption spectrum as rifampicin, it could not be differentiated from rifampicin (Brechbühler *et al*, 1978). The spectroscopically determined sum of rifampicin and desacetyl metabolite was referred to as apparent rifampicin.

Rifampicin in urine was almost completely extracted by using a mixture solvent consisting of an equal volume of benzene and hexane, since extraction of desacetylrifampicin in the solvent is negligible (Sunahara *et al*, 1972). On this principle, the urine sample which was treated with the benzene-hexane mixture, was re-extracted with the previous method and the absorption maximum was obtained for the metabolites.
The concentration of unchanged rifampicin in the urine sample was calculated as the difference in value between the toluene extracts containing the apparent rifampicin and the toluene extract containing the metabolite.

Results

The results were expressed as mean ±SEM and an analysis of variance (ANOVA), employing the PC-90 version of BMDP for IBM computers and compatables, was applied for statistical purpose. $P < 0.05$ was considered statistically significant.

A. Saliva

Table 1 & 2 show the mean salivary rifampicin levels at different time intervals after the single oral dose of 600mg of both Treatment A (Standard drug manufactured by Ciba-Geigy) and Treatment B (Test drug manufactured by Pharmamed) was administered to male and female volunteers respectively.

Male Volunteers				
Time (hrs)	Treatment A Mean (µg/ml)	±SEM	Treatment B Mean (µg/ml)	± SEM
0	0.000	0.000	0.000	0.000
1	0.089	0.078	0.165	0.088
2	1.625	0.905	0.259	0.102
4	0.222	0.085	0.188	0.124
6	0.134	0.075	0.163	0.181
8	0.076	0.045	0.089	0.108
12	0.026	0.020	0.018	0.020
24	0.001	0.000	0.001	0.000

Table 1. Mean Salivary Rifampicin Levels at different time intervals after the oral dose of Treatments A & B in male volunteers.

Female Volunteers				
Time (hrs)	Treatment A Mean (µg/ml)	±SEM	Treatment B Mean (µg/ml)	± SEM
0	0.000	0.000	0.000	0.000
1	0.203	0.122	0.264	0.169
2	0.605	0.263	0.346	0.127
4	0.482	0.198	0.368	0.132
6	0.301	0.136	0.216	0.090
8	0.154	0.070	0.116	0.064
12	0.041	0.020	0.029	0.020
24	0.003	0.000	0.001	0.000

Table 2. Mean Salivary Rifampicin Levels at different time intervals after the oral dose of Treatments A & B in female volunteers.

The graphical representations showing the mean salivary rifampicin concentrations obtained at different time intervals in male and female volunteers, are given in Figures 1 & 2 respectively.

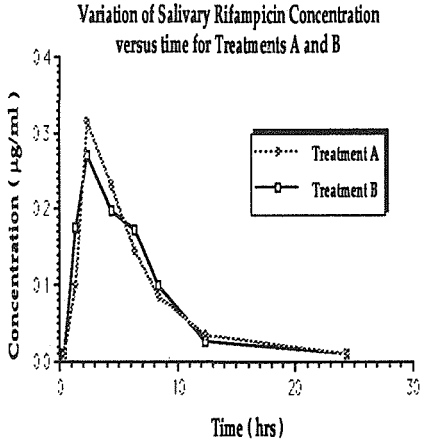


Figure 1. Mean Salivary Rifampicin Levels at different time intervals after the oral dose of Treatments A & B in 12 male volunteers.

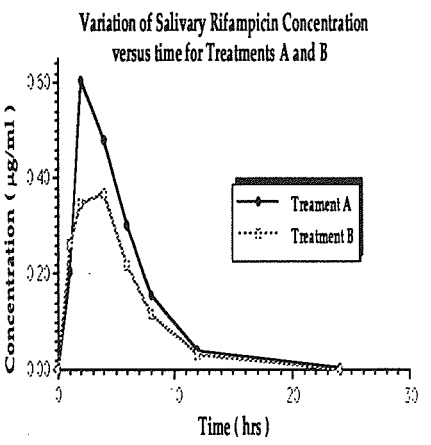


Figure 2. Mean Salivary Rifampicin Levels at different time intervals after the oral dose of Treatments A & B in 12 female volunteers.

Table 3 & 4 give the mean ±SEM for the three pharmacokinetic parameters which were considered, ie. C_{max}, T_{max}, and AUC, as observed in the male and female volunteers respectively.

Table 3.
The means of the the three pharmacokinetic parameters investigated following the oral administration of Treatments A & B in male volunteers.

Male Volunteers						
Volunteers	Treatment A			Treatment B		
	C_{max} (ng/ml)	t_{max} (hrs)	AUC (ng/ml.hrs)	C_{max} (ng/ml)	t_{max} (hrs)	AUC (ng/ml.hrs)
AF	0.800	2	3.799	1.057	6	7.493
AA	0.195	2	0.752	0.219	4	1.062
AS	0.175	4	0.705	0.084	4	0.563
CS	0.201	4	1.659	0.537	2	2.298
HA	0.120	6	0.839	0.202	2	0.847
IM	0.093	4	0.536	0.138	2	0.716
IM	0.594	2	1.477	0.195	1	1.832
IM	1.153	2	4.838	0.433	2	1.251
MM	0.261	4	1.187	0.253	2	0.687
WB	0.253	4	1.069	0.439	1	1.626
CF	0.142	4	0.903	0.178	2	0.682
SS	0.313	2	2.654	0.140	1	1.037
Mean	0.356	3.143	1.702	0.329	2.417	1.678
± SEM	0.297	0.828	0.467	0.172	0.956	1.211

Table 4.
The means of the the three pharmacokinetic parameters investigated following the oral administration of Treatments A & B in female volunteers.

Female Volunteers						
Volunteers	Treatment A			Treatment B		
	C_{max} (ng/ml)	t_{max} (hrs)	AUC (ng/ml.hrs)	C_{max} (ng/ml)	t_{max} (hrs)	AUC (ng/ml.hrs)
AB	0.923	2	4.769	0.592	4	3.805
AM	0.622	2	2.543	0.252	2	1.260
CC	0.378	4	1.742	0.223	2	1.117
CZ	0.783	2	3.145	0.526	1	2.694
CM	0.439	1	2.550	0.560	4	3.592
EA	0.276	4	1.451	0.291	6	1.805
HD	0.458	6	1.956	0.172	4	1.023
MP	1.322	2	5.756	0.576	4	2.277
MB	1.098	2	4.301	0.691	2	2.509
NP	1.294	4	7.532	0.761	4	5.008
MP	0.519	4	2.937	0.626	2	3.174
MM	0.698	4	3.282	0.828	1	2.291
Mean	0.734	3.083	3.497	0.508	3.000	2.646
± SEM	0.226	0.917	1.142	0.140	0.977	0.763

On comparing the mean values for every pharmacokinetic parameter in Treatments A and B it was noted that the means for Treatment B were slightly lower than those for Treatment A. However, no statistically significant difference in the calculated mean (±SEM) value of the pharmacokinetic parameters C_{max}, T_{max}, and AUC for both male and female volunteers could be detected between the two treatments.

B. Urine

Tables 5 & 6 show the amount of drug excreted at infinite time, Ae^{∞} for both treatments in male and female volunteers respectively.

Male Volunteers				
Time	Treatment A		Treatment B	
	Mean Ae^{∞} (mg)	± SEM	Mean Ae^{∞} (mg)	± SEM
0	0.000	0.000	0.000	0.000
1	2.594	2.980	1.830	0.756
2	9.430	3.319	8.361	2.527
4	22.535	3.768	20.887	3.978
6	33.384	4.707	29.337	5.986
8	44.164	5.179	37.043	6.196
12	52.358	7.785	45.654	7.351
24	60.715	10.453	50.501	9.024
28	60.915	10.453	51.128	9.032
30	60.915	10.453	51.128	9.032
32	60.915	10.453	51.128	9.032

Table 5. The mean amounts of drug excreted unchanged at different time intervals after the oral administration of Treatments A & B in male volunteers.

Female Volunteers				
Time	Treatment A		Treatment B	
	Mean Ae^{∞} (mg)	± SEM	Mean Ae^{∞} (mg)	± SEM
0	0.000	0.000	0.000	0.000
1	0.539	1.531	1.429	1.226
2	5.919	2.177	6.081	3.201
4	20.997	3.630	17.832	4.902
6	34.987	4.751	30.995	5.303
8	49.146	6.488	41.106	7.388
12	59.739	8.831	48.024	8.058
24	71.596	10.233	55.350	10.140
28	72.405	10.242	55.654	10.130
30	72.405	10.242	55.654	10.130
32	72.405	10.242	55.654	10.130

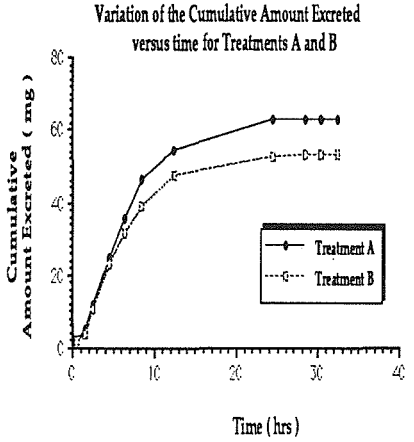
Table 6. The mean amounts of drug excreted unchanged at different time intervals after the oral administration of Treatments A & B in female volunteers.

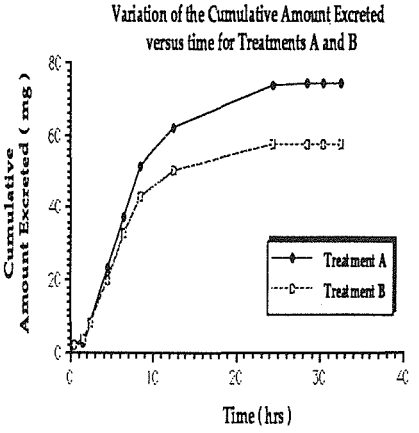
Table 7 shows the amount of drug excreted at infinite time, Ae^{∞} for both treatments in male and female volunteers respectively.

Male Volunteers			Female Volunteers		
Volunteers	Treatment A Ae^{∞} (mg)	Treatment B Ae^{∞} (mg)	Volunteers	Treatment A Ae^{∞} (mg)	Treatment B Ae^{∞} (mg)
AF	83.111	70.611	AB	90.951	60.216
AA	55.916	27.417	AM	39.293	49.506
AS	58.044	47.001	CC	75.083	49.300
CS	63.767	60.844	CZ	66.741	38.876
HA	34.854	44.682	CM	85.891	89.429
IM	72.972	75.097	EA	75.695	44.585
IM	28.178	43.366	HD	82.753	71.319
IM	71.286	40.916	MP	82.786	30.931
MM	54.368	41.291	MB	54.536	46.946
WB	58.670	47.211	NP	83.274	69.547
CF	74.814	67.689	MP	50.931	59.208
SS	75.002	49.408	MM	80.921	58.390
Mean	60.919	53.754	Mean	72.408	58.624
± SEM	10.433	9.022	± SEM	10.342	10.230

Table 7. The mean amounts of drug excreted unchanged at infinite time Ae^{∞} for Treatments A & B in both male and female volunteers.

Figures 3 & 4 show a graphical representation of the mean cumulative urinary excretion of unchanged drug for both treatments in male and female volunteers respectively.





Both means for the test drug were lower than the corresponding means for the standard drug in both males and females. There was no statistically significant difference between the means of drug excreted unchanged after Treatment A and Treatment B in males. However, this difference was statistically significant in females.

Conclusion

The present study was undertaken to compare the oral bioavailability of rifampicin from a new combined formulation with that of a widely prescribed brand.

The results obtained document the comparable bioavailability of both drug formulations. However, there is an indication of some variation between the results obtained in males and female. This imparts a reduced clarity about the precise significance of classical bioequivalence testing, which is carried out on healthy young male subjects only, since such studies may not represent the action of the drug in the whole population.

The results also showed that in all the parameters measured, the test drug results were slightly lower than those for the standard drug. This can be attributed to the characteristics of solid state and physical properties of the rifampicin molecule, which have been studied extensively to ascertain their influence on the bioavailability of the drug. Rifampicin has been shown to exist in two polymorphic forms as a single entity, in several solvates and in an amorphous phase. Its crystalline habit can be changed as a consequence of the manufacturing procedure used in the preparation of the dosage form.

In studies carried out by Cavenaghi, the results showed that variations in particle size, excipients or manufacturing process may result in a marked decrease in the absorption of rifampicin (Cavenaghi, 1989). Therefore, modifications in the pharmaceutical properties andmanufacture process of the generic drug may be necessary in order to approach even more the bioavailability profile of the standard product.

References

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